by A. Lihme and T. Boenisch ("Water soluble, polymer based reagents and conjugates comprising moieties derived from divinely sulfone", WO 93/01498, ref. 22) resulting in a degree of vinylsulfone activation of approximately 25% of the monomer units.

FITC-streptavidine-dextran (150, 270, 500 kDa) conjugates

Streptavidine (SA, Genzyme) was dialysed overnight (100 mg in 5 ml, against 1000 ml 0.10 M NaCl, 2-4°C, 10 kDa MwCO, changed three times).

A fluorescein isothiocyanate (FITC, Molecular Probes) solution (14.0 mg/ml DMF) was added to a stirred mixture of streptavidine (14.0 mg SA/ml, 0.19 mg FITC/ml, 0.1 M NaCl, 25 mM carbonate buffer, pH 8.5, 30°C).

After 6 hours, the reaction mixture was added to a solution of vinylsulfon-activated dextran (approximately 25% activated) of 150, 270 or 500 kDa (in total 1.6 mg vinylsulfon dextran/ml, 7.7 mg SA/ml, 0.1 M NaCl, 25 mM carbonate buffer, pH 8.5) and stirred at 30°C 18 hours.

Any remaining reactive groups were quenched by addition of 1/10 volume reaction mixture of an ethanolamine-containing buffer (110 mM ethanolamine, 50 mM HEPES, 0.1 M NaCl, pH 7.0) and stirred for 30 minutes at 30°C.

The obtained polymeric conjugate was purified from free fluorescein and unbound streptavidine by gelfiltration (FPLC, Pharmacia, S-200, 0.1 M HEPES, 0.1 M NaCl, pH 7.2).

The degree of fluorescein and streptavidine incorporation could be calculated from the UV absorbance at 278 and 498 nm in the three fractions containing conjugate, unbound streptavidine and unbound fluorescein, respectively. The

conjugates were added sodium azide to 15 mM as a preservative.

Dextran carrier molecule	SA per dextran	FITC per SA	Concentration dextran (mole/l)
150	4.4	2.7	61.1×10^{-8}
270	6.9	2.6	54.7×10^{-8}
500	13.6	2.7	31.2×10^{-8}

Unless otherwise stated the FITC conjugated 500, 270 and 150 kDa dextrans used in examples described below were conjugated in average with about 13.6 (in the case of the 500 kDa dextran), 6.9 (in the case of the 270 kDa dextran) and 4.4 (in the case of the 150 kDa dextran) SA complexes per dextran molecule.

Preparation of HRP-streptavidine-dextran (70, 150, 270 kDa) conjugates

Horseradish peroxidase (HRP, Fairzyme) and streptavidine (SA, Genzyme) were dialysed overnight (100 mg in 5 ml, against 1000 ml 0,10 M NaCl, 2-4°C, 10 kDa MwCO, changed three times) before being concentrated. The conjugation was performed by sequential addition of HRP and streptavidine to activated dextran.

The HRP solution was added to a solution of vinylsulfon activated dextran (approximately 25% activated) of 70, 150 or 270 kDa (totally 40.0 mg HRP/ml, 1.6 mg dextran/ml, 25 mM carbonate, 0.1 M NaCl, pH 8.5) and stirred on a water bath (30°C, 6.0 hours). The streptavidine solution was added to the reaction mixture (totally 9.14 mg streptavidine/ml, 1.06 mg dextran/ml, 26.67 mg HRP/ml 25 mM carbonate, 0.10 M NaCl, pH 8.5) and stirred overnight on a water bath (30°C, 18 hours).

Any remaining reactive groups were quenched by addition of 1/10 volume reaction mixture of an ethanolamine-containing buffer (110 mM ethanol amine, 50 mM HEPES, 0.1 M NaCl, pH 7.0) and stirred for 30 minutes at 30°C.

The conjugate was separated from unconjugated streptavidine and HRP by gelfiltration (FPLC, Pharmacia, S-200, 0.1 M HEPES, 0.1 M NaCl, pH 7.2).

The degree of HRP and streptavidine incorporation could be calculated from the UV absorbance at 280 and 403 nm of the fraction containing the conjugate and the fraction containing streptavidine and HRP. The conjugates were added BSA as protein stabiliser and bronidox as preservative.

Dextran carrier molecule	SA per dextran	HRP per dextran	Concentration dextran (mole/l)
70	3.0	2.3	10.5×10^{-8}
150	5.4	3.7	7.0×10^{-8}
270	8.0	5.3	4.6×10^{-8}

B. Production of peptide-loaded MHC molecules

The HLA Class I heavy and light (β_2m) chains were produced and partially purified as inclusion bodies from an E.coli strain (BL21 (DE3), Novagen (Novagen, Inc, Madison, WI, USA) following standard procedure.

The isolated inclusion body molecules were solubilised in 8M urea at non-reducing conditions to obtain heavy chain molecule with intact disulphide bonds. The heavy chain molecule was additionally purified by size- and ion-exchange chromatography following standard procedure and finally subjected to folding as described below.

Peptide epitope specific HLA Class I complexes were generated in vitro using a "folding by dilution" approach where the higly purified preparations of denatured HLA Class I heavy chain molecule (about 10-20 µM in 8M urea) A0201, 1-275) were renatured by incubation in a 100-fold dilution buffer (final concentration of heavy chain is thus about 100-200 nM) containing the peptide of interest (10 µM) and β_2m (1 µM), for 16 hours at 18°C. Misfolded HLA Class I heavy chain was precipitated by centrifugation prior to purification of *de novo* folded HLA Class I molecule by G75 size exclusion chromatography following standard procedure. The fraction of folded HLA A0201 molecule was ruinously about 40-50% of total amount of HLA A0201 heavy chain molecule added to the folding reaction. The fraction of misfolded heavy chain molecule contained inappropriate disulphide bonds and was not available for renaturation. This folding scheme, described above, was useful for rapid generation of a variety of peptide-loaded monomer MHC Class I complexes encoded by the polymorphic HLA and H-2 gene complexes. The purified complexes were finally enzymatic mono-biotinylated utilising protein ligase BIR A as described by the manufacturer (AVIDITY; Denver, Co, USA).

C. Production of peptide empty HLA Class I molecules

Peptide empty MHC Class I was produced in a process where functional mono-biotinylated MHC Class I complexes (cf. example 1B) initially were denatured by addition of urea (8M) or guanidine (6M). The chaeotrophic buffers dissociated the structural molecule subunits from the Class I complex, leaving free soluble biotinylated heavy chain and free soluble β_2m molecules available for biochemical purification. The heavy chain molecule was excluded from the dissociated β_2m and peptide by G75 size exclusion chromatography following standard procedure. The purified heavy chain molecule form spontaneously a

peptide receptive hetero-dimer complex consisting of heavy and light chain in a folding buffer containing excess β_2m (cf. Example 1:B hereabove). The peptide empty HLA Class I dimer remained stable in excess of β_2M and could be ligated to streptavidin to form peptide empty construct of the invention or peptide empty tetramer. Peptide (1 µM) of interest were be added to during or after the process of ligation with soluble or SA-conjugated dextran to generate TCR-binding MHC molecules in the form of MHC molecule constructs of the invention or MHC molecule tetramers.

D. Production of poly-ligand MHC molecule constructs of the invention

The preparations of SA conjugated dextrans of different molecular sizes were mixed with amounts of HLA complexes corresponding to a ratio of two biotinylated HLA Class I molecules per SA molecule. The HLA molecule was added directly to a solution of SA-conjugated dextrans. Thus, MHC molecule constructs were formed comprising

(1) a carrier molecule being a 500 kDa dextran having attached thereto 27.2 biotinylated HLA Class I molecules (MHC molecules) via about 13.6 FITC-labelled SA (binding entities) (in average 2 HLA Class I molecules per SA), each SA labelled in average with 2.7 FITC,

(2) a carrier molecule being a 270 kDa dextran having attached thereto about 13.8 biotinylated HLA Class I molecules (MHC molecules) via about 6.9 FITC-labelled SA (binding entities) (in average 2 HLA Class I molecules per SA), each SA labelled in average with 2.6 FITC,

(3) a carrier molecule being a 150 kDa dextran having attached thereto about 8.8 biotinylated HLA Class I molecules (MHC molecules) via about 4.4 FITC-labelled SA

(binding entities) (in average 2 HLA Class I molecules per SA), each SA labelled in average with 2.7 FITC,

(4) a carrier molecule being a 70 kDa dextran having attached thereto about 6.0 biotinylated HLA Class I molecules (MHC molecules) via about 3.0 SA (binding entities) (in average 2 HLA Class I molecules per SA), each dextran labelled in average with 2.3 HRP enzymes,

(5) a carrier molecule being a 270 kDa dextran having attached thereto about 10.8 biotinylated HLA Class I molecules (MHC molecules) via about 5.4 SA (binding entities) (in average 2 HLA Class I molecules per SA), each dextran labelled in average with 3.7 HRP enzymes,

(6) a carrier molecule being a 270 kDa dextran having attached thereto about 16.0 biotinylated HLA Class I molecules (MHC molecules) via about 8.0 SA (binding entities) (in average 2 HLA Class I molecules per SA), each dextran labelled in average with 5.3 HRP enzymes.

The attachment of this high number of HLA Class I molecules was possible due to the high affinity between SA and biotin (affinity dissociation constant; $K_D = 10^{15}$).

By this procedure, the following MHC molecule constructs of the present invention were prepared:

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 biotinylated HLA A0201 in complex with the MART-1 peptide analogue (ELAGIGILTV) and β_2m via 13.6 FITC labelled SA (in average 2 HLA A0201 molecules per SA, and in average 2.7 FITC per SA) (MHC molecule construct 1),

a MHC molecule construct comprising the 270 kDa dextran carrier molecule having attached thereto 13.8 biotinylated HLA A0201 molecules in complex with the MART-1 peptide analogue (ELAGIGILTV) and β_2m via 6.9 FITC labelled SA (in average 2 HLA A0201 molecules per SA, and in average 2.6 FITC per SA) (MHC molecule construct 2),

a MHC molecule construct comprising the 150 kDa dextran carrier molecule having attached thereto 8.8 biotinylated HLA A0201 molecules in complex with the MART-1 peptide analogue (ELAGIGILTV) and β_2m via 4.4 FITC labelled SA (in average 2 HLA A0201 molecules per SA, and in average 2.7 FITC per SA) (MHC molecule construct 3),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 biotinylated HLA A0201 in complex with the influenza matrix protein amino acids 58-66 (GILGFVFTL) and β_2m via 13.6 FITC labelled SA (in average 2 HLA A0201 molecules per SA and in average 2.7 FITC per SA) (MHC molecule construct 4),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 biotinylated HLA A0201 in complex with the wild type P53 peptide R9V (RMPEAAPPV) and β_2m via 13.6 FITC labelled SA (in average 2 HLA A0201 molecules per SA and in average 2.7 FITC per SA) (MHC molecule construct 5),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 biotinylated HLA A0201 in complex with the wild type P53 peptide G11V (GLAPPQHLIRV) and β_2m via 13.6 FITC labelled SA (in average 2 HLA A0201 molecules per SA and in average 2.7 FITC per SA) (MHC molecule construct 6),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 biotinylated peptide empty HLA A0201 via 13.6 FITC labelled SA (in average 2 HLA A0201 molecules per SA and in average 2.7 FITC per SA) (MHC molecule construct 7),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 biotinylated HLA A0201 in complex with the gp100 peptide KTWGQYWOV and β_2m via 13.6 FITC labelled SA (in average 2 HLA A0201 molecules per SA and in average 2.7 FITC per SA) (MHC molecule construct 8),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 HLA A0201 heavy chain in complex with the MART-1 peptide analogue (ELAGIGILTV) and iodinated β_2m via 13.6 SA (in average 2 HLA A0201 molecules per SA), having a radioactivity of 100000 cpm/sample) (MHC molecule construct 9),

a MHC molecule construct comprising the 270 kDa dextran carrier molecule having attached thereto 16.0 biotinylated HLA A0201 in complex with the Mart-1 peptide analogue (ELAGIGILTV) and β_2m via 8.0 SA (in average 2 HLA A0201 molecules per SA) and 5.3 HRP enzymes to the dextran (MHC molecule construct 10),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 biotinylated HLA A0201 in complex with the sur1/M2 peptide analogue (LMLGEFLKL) and β_2m via 13.6 FITC labelled SA (in average 2 HLA A0201 molecules per SA, and in average 2.7 FITC per SA) (MHC molecule construct 11),

a MHC molecule construct comprising the 150 kDa dextran carrier molecule having attached thereto 10.8 biotin-

ylated HLA A0201 in complex with the MART-1 peptide analogue (ELAGIGILTV) and β_2m via 5.4 SA (in average 2 HLA A0201 molecules per SA) and 3.7 HRP enzymes to the dextran (MHC molecule construct 12),

a MHC molecule construct comprising the 70 kDa dextran carrier molecule having attached thereto 16.0 biotinylated HLA A0201 in complex with the MART-1 peptide analogue (ELAGIGILTV) and β_2m via 3.0 SA (in average 2 HLA A0201 molecules per SA) and 2.3 HRP enzymes to the dextran (MHC molecule construct 13),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 biotinylated HLA A0201 in complex with the MAGE-3 peptide (FLWGPRALV) and β_2m via 13.6 FITC labelled SA (in average 2 HLA A0201 molecules per SA and in average 2.7 FITC per SA) (MHC molecule construct 14),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 14.1 biotinylated HLA A0201 in complex with the MART-1 peptide analogue (ELAGIGILTV) and β_2m via 13.6 FITC labelled SA (in average 1 HLA A0201 molecules per SA, and in average 2 FITC per SA) (MHC molecule construct 15),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 14.1 biotinylated HLA A0201 in complex with the MART-1 peptide analogue (ELAGIGILTV) and β_2m and 7.1 MIC A molecules via 13.6 FITC labelled SA (in average 1 HLA A0201 molecules per SA, and in average 2 FITC per SA) (MHC molecule construct 16),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 14.1 biotin-

ylated HLA A0201 in complex with the gp100 peptide KTWGQYWOV and β_2m via 13.6 FITC labelled SA (in average 1 HLA A0201 molecules per SA and in average 2 FITC per SA) (MHC molecule construct 17),

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 14.1 biotinylated HLA A0201 in complex with the gp100 peptide KTWGQYWOV and β_2m and 7.1 MIC A molecules via 13.6 FITC labelled SA (in average 1 HLA A0201 molecules per SA and in average 2 FITC per SA) (MHC molecule construct 18).

EXAMPLE 2

Production of MHC molecule tetramers

The peptide epitope specific HLA molecule used for the tetramers was generated as described in Example 1, B. The tetramers were formed by sequential addition of small amounts of PE-conjugated SA (Molecular Probes, Holland) to a solution of biotinylated HLA complexes. The final amount of HLA complex in the mixture should be four-fold the amount of SA to ensure saturation (four biotin binding sites per SA complex).

By this procedure, the following tetramers were prepared:

A PE-labelled tetramer consisting of four biotinylated HLA A0201 in complex with the modified MART-1 peptide (ELAGIGILTV) and β_2m (tetramer 1),

a PE-labelled tetramer consisting of four biotinylated HLA A0201 in complex with the gp100 peptide (KTWGQYWOV) (tetramer 2) and β_2m,

a PE-labelled tetramer consisting of four biotinylated HLA A0201 in complex with the influenza matrix protein amino acids 58-66 (GILGFVFTL) (tetramer 3),

a PE-labelled tetramer consisting of four biotinylated HLA A0201 in complex with the wild type P53 peptide R9V (RMPEAAPPV) (tetramer 4),

a PE-labelled tetramer consisting of four biotinylated HLA A0201 in complex with the wild type P53 peptide G11V (GLAPPQHLIRV) (tetramer 5),

a PE-labelled peptide empty tetramer consisting of four PE-labelled peptide empty HLA A0201 (tetramer 6).

EXAMPLE 3

Dose dependent binding of MHC molecule constructs according to the invention as compared to MHC molecule tetramers to the T-cell is peptide specific

In this experiment, the binding of peptide epitope specific MHC molecule constructs of the invention and MHC molecule tetramers to established T-cell clones was investigated.

Previously established and characterised "in house" T-cell clones, named 5/127 and 5/130, which reacted against melanoma specific tumour antigens, were utilised to analyse binding of the HLA molecule constructs (i.e. MHC molecule constructs) of the invention to TCR on cell surfaces by flow cytometry following a standard flow cytometry protocol. Briefly, 5×10^5 cells were incubated in 50 µl "FACS-buffer" (phosphate-buffered saline (PBS), 10 mg/ml bovine serum albumin (BSA), 0.2% azide) with either the poly-ligand MHC molecule constructs of the

invention or the tetramers, displaying the peptides of interest. Unless otherwise stated, the cells were washed once in the FACS buffer and analysed on a Becton Dickenton FACSCalibur flow cytometer.

The two T-cell clones reacted specifically with HLA A0201 bound peptides from the tumour (melanoma)-associated antigens MART-1 and gp100, respectively.

The following poly-ligand HLA molecule constructs of the invention were used:

MHC molecule construct 1,
MHC molecule construct 2,
MHC molecule construct 3.

The following MHC molecule tetramers were used:

tetramer 1,
tetramer 2.

The PE-labelled tetramers 1 and 2 were used for comparison.

The T-cell clones 5/127 and 5/130 were thawed and grown 24 hours at 37°C in presence of 50U IL-2 and 10% human serum. About 5×10^5 T-cell clones were incubated 1 hour at 22°C with graded doses of MHC molecule construct of the invention (MHC molecule construct 1: 0-9.36 nM, 2-fold dilutions, cf. Figure 25; MHC molecule construct 2: 0-27.5 nM, 2-fold dilutions, cf. Figure 25; MHC molecule construct 3: 0-37.5 nM, 2-fold dilutions, cf. Figure 25) or PE-labelled tetramers (tetramers 1 and 2; 0-200 nM, 2-fold dilutions, cf. Figure 25). After incubation, the cells were washed only once to avoid dissociation of low avidity bound MHC molecule constructs or tetramer, and

analysed by flow cytometry following standard flow cytometry procedures for cell bound MHC molecule construct (results shown in Figure 25B) and PE-labelled tetramer (results shown in Figure 25A).

The MART-1 peptide specific T-cell clone 5/127 (indicated as squares in Figure 25A) bound tetramers that displayed ELAGIGILTV peptide (open squares) with high avidity. Half-maximal staining of the 5/127 T-cells was observed by addition of 20-30 nM of tetramers. In the control experiment, the tetramer preparation that displayed the gp100 peptide KTWGQYWOV (filled squares) did not interact with the 5/127 T-cell clones. In comparison, the gp100 reactive T-cell clone 5/130 was stained with tetramers displaying the gp100 peptide KTWGQYWOV (black circles) and interacted only weakly with high concentrations of tetramers displaying the ELAGIGILTV peptide (open circles). The binding of peptide specific tetramers to the two T-cell lines showed that about 100 nM tetramers almost saturated the 5/127 cell line, whereas the 5/130 cell line was only partially stained due to low avidity binding. Though the peptide specific tetramer preparations clearly bound with different avidity, the data demonstrated clearly that both cell lines bound appropriate peptide-HLA complexes specifically. Thus, it was concluded that both T-cell clones were useful for analysis of the constructs of the invention.

For the subsequent analyses of the binding of different construct of the invention and for with comparison the tetramer constructs, the robust 5/127 T-cell clones were chosen.

The T-cell clone 5/127 was stained as described above with MHC molecule constructs 1, 2 and 3 of the invention. As shown in Figure 25B, all sizes of dextran carrier

molecules facilitated a dose dependent staining of the MART-1 specific T-cell clone. In comparison, the larger construct (the 500 kDa dextran carrier molecule) bound more efficiently to the T-cells than the intermediate construct (270 kDa dextran carrier molecule) and the smaller construct (150 kDa dextran carrier molecule). However, as evident from dose dependent staining of the cells shown in Figure 25A, all three constructs stained the 5/127 T-cell clone more efficiently than did the tetramers which had to be added in higher amounts to obtain significant staining of the cells (compare Figure 25A (open squares) with the three curves in Figure 25B). The improved binding avidity of the three constructs of the invention was clearly reflected by the low concentrations of the constructs (2-10 nM) required for half-saturation, whereas the corresponding tetramers required 20-30 nM for half-saturation (cf. the tetramer staining in Figure 25A).

Thus, it was concluded that the constructs of the invention bound dose-dependent to peptide epitope specific T-cells and with higher avidity than corresponding tetramers displaying identical peptides.

EXAMPLE 4

Binding of MHC molecule constructs of the invention and tetramers to influenza specific T-cell line

In this experiment, the binding of peptide specific constructs of the invention and tetramers to a T-cell line recognising a conventional non-self peptide presented in context of HLA A0201 molecules was investigated.

Dendritic cells (DC) were generated from freshly isolated PBMC from HLA-A0201 donors following standard protocols, using 250 U/ml hrIL-4 (R&D Systems, Minneapolis, MN, USA) and 500 U/ml hrGM-CSF (Leucomax, Novartis/Schering-Ploug, Basel, Switzerland) for DC culture and 72 hours exposure to hrCD40LT, 1 μg/ml (Immunex Corporation, Seattle, Washington, USA) to induce DC maturation.

On day 10 of culture, DC were isolated by EDTA treatment and loaded with the influenza peptide IMP 58-66 (40 μg/ml) for 1 hour followed by wash and irradiation (3000 Rad). Subsequently, freshly isolated autologous PBMC (2×10^6/ml) were added to the peptide loaded DC (1-2×10^5/ml) in 24 well plates in 1 ml AB-medium/well containing 20 U/ml rhIL-4 and 5 ng/ml rhIL-7 (Peprotech EC, London, UK). After 9-11 days, T-cell cultures were depleted for CD4+ cells by Dynabead® separation (according to the manufacturers instructions) and the negatively selected CD8+ cells (4×10^5/ml) were re-stimulated with peptide pulsed autologous irradiated DC (1-2×10^5/ml) and irradiated (3000 Rad) autologous PBMC (10^6/ml) in AB-medium supplemented with rhIL-4 and rhIL-7 in 96 wells U-bottomed plates. Further re-stimulations were performed every 7th day of culture as described above using irradiated (6000 Rad) peptide pulsed HLA-$A2^+$ EBV-B-cells (2×10^5/ml) as stimulators and irradiated (3000 Rad) allogeneic PBMC. rhIL-2 (20 U/ml, Proleukin, Chiron, CA, USA) was added at day 1 after each re-stimulation.

The following MHC molecule constructs of the invention were used:

MHC molecule construct 4,
MHC molecule construct 5,
MHC molecule construct 6.

The following tetramers were used:

tetramer 3,
tetramer 4,
tetramer 5.

The tetramers 3-5 were used for comparison.

5×10^5 T-cells were incubated in 50 µl FACS-buffer (PBS, 10 mg/ml BSA, 0.2% azide) with the poly-ligand MHC molecule constructs of the invention or the tetramers, all displaying peptides of interest.

The cells were incubated for 90 minutes at 22°C in graded doses of the constructs of the invention (0-32 nM, 2-fold dilutions, cf. Figure 26) or the tetramers (0-112 nM, 2-fold dilutions, cf. Figure 26), washed once and analysed by flow cytometry following standard flow cytometry procedures for cell bound molecule construct of and tetramer, respectively.

As shown in Figure 26, the fraction of peptide specific T-cells (about 55%) in the established cell line was fully stained using low concentrations of the constructs of the invention (<30 nM), whereas the tetramers stained the cells less efficiently. Half-maximal staining was obtained with about 3 nM of the constructs of the invention and 30 nM of the tetramers. In contrast, the constructs of the invention and tetramers expressing wild-type P53 peptides did not stain the T-cells. Thus, it was concluded that the constructs of the invention stained sub-populations of influenza specific T-cells specifically and with higher efficacy than the peptide identical tetramers. As illustrated in Figure 25 and 26, the improved staining efficiency was produced by the

higher HLA molecular valence of the constructs as compared to the tetramers.

EXAMPLE 5

Time dependence of MHC molecule construct and tetramer binding

In this experiment, it was shown that binding of MHC molecule constructs of the invention appear to be time dependent. The results obtained are shown in Figure 27. For comparison, PE-labelled tetramers displaying the same peptides as the used MHC molecule constructs of the invention were tested in parallel assays.

The following MHC molecule construct of the invention was used:

MHC molecule construct 1.

The following tetramer was used:

Tetramer 1.

Briefly, 5×10^5 MART-1 specific T-cell cones (5/127) were incubated in 50 μl FACS-buffer (PBS, 10 mg/ml BSA, 0.2% azide) with the poly-ligand MHC molecule constructs of the invention or the tetramers, all displaying peptides of interest. The T-cell clones were incubated in graded doses (2-fold dilutions, cf. Figure 27) of the construct of the invention or the tetramer, both displaying the MART-1 related peptide analogue ELAGIGILTV. The cells were incubated at room temperature (22°C). Aliquots of cells were taken at different time points (cf. Figure 27) washed and measured by flow cytometry following standard procedures for flow cytometry for cell bound construct

(shown in Figure 27B) or tetramer (shown in Figure 27A). As shown in Figure 27A, in case of the tetramer, a steady-state binding was obtained after 1 hour of incubation using high concentrations of tetramer (112 nM), whereas lower concentrations (14-56 nM) did not reach steady-state within the measured time interval. In comparison, the construct of the invention reached a steady-state level within 60 minutes using a significant lower concentration of construct (16 nM).

Thus, it was demonstrated that the association of the constructs of the invention was faster than association of the tetramers, presumably due to a higher valence of the constructs of the invention.

EXAMPLE 6

Dissociation of cell bound constructs of the invention

In this experiment, the dissociation of cell bound constructs of the invention was investigated.

The following MHC molecule construct was used:

MHC molecule construct 1.

T-cell clones (5/127) were incubated with the construct of the invention displaying the MART-1 related peptide analogue ELAGIGILTV. The cells (5×10^5) were incubated 1 hour at 22°C and washed once and incubated at 4°C, 22°C and 37°C, respectively, in FACS-buffer (PBS, 10 mg/ml BSA, 0.2% azide) containing 50 nM CD8 specific monoclonal antibody to prevent re-binding of dissociating construct. At different time points (0, 60, 90, 120 minutes, respectively), aliquots of cells were taken, washed and analysed for cell bound constructs by flow cytometry

following a standard protocol for flow cytometry. The results are shown in Figure 28. At 4°C, the half-life of the construct binding was about 90 minutes, which were reduced to about 50 and 30 minutes at 22°C and 30°C, respectively. The biphasic dissociation of the constructs from cells incubated at 37°C indicated that some degree of internalisation of the construct into the cells took place. Alternatively, biphasic dissociation could explained with complex interaction between the construct and counter receptors on the T-cell surface at 37°C as compared to binding of the same construct at lower temperatures.

Thus, it was concluded that dissociation of cell bound construct was time and temperature dependent.

EXAMPLE 7

Binding of construct of the invention: the impact of antibodies

In this experiment, it was shown that cell surface binding of constructs of the invention is affected by HLA Class I specific monoclonal antibodies reacting with HLA Class I epitopes in close proximity of the peptide-binding site.

The following MHC molecule construct of the invention was used:

MHC molecule construct 1.

The following monoclonal antibodies were used:

BB7.2 (HLA A0201 specific), W6/32 (HLA A,B,C pan specific), BBM1 (human β_2m specific), and mouse anti

human T-cell, CD8 Clone DK25 (DAKO Code No. M0707) (CD8-specific antibody).

The MART-1 specific T-cell clone 5/127 was incubated with a mixture of 2 nM construct displaying the MART-1 related peptide epitope with or without 10 nM monoclonal antibody (BB7.2, W6/32, BBM1 and CD8 specific, respectively) as indicated in Figure 29A. The cells were incubated for 90 minutes at 22°C, washed once and analysed flow cytometry following standard procedures for cell bound construct. The monoclonal antibodies B7.1 and W6/32 that reacted with epitopes in close proximity of the peptide binding site of HLA A0201 inhibited as shown in Figure 29A the binding of the construct to a level near the background (the background signal being obtained by incubating cells with 10 nM FITC-labelled construct with no HLA molecules).

In contrast, the presence of monoclonal antibody BBM1 that bound to the HLA Class I light chain, β_2m, did not affect the binding of the construct.

In a similar experiment (cf. Figure 29B), the impact of a CD8 specific monoclonal antibody was analysed. The T-cells (5/127) were incubated for 60 minutes at 22°C with the MHC molecule construct 1 (cf. Figure 29A) and graded doses of antibody 0-12 nM (cf. Figure 29B). The cells were washed and analysed by flow cytometry following standard flow cytometry procedures for cell bound construct. The CD8 specific antibody strongly inhibited the association of the construct to the T-cells, cf. Figure 29B, suggesting that CD8 molecules on the T-cells contribute significantly to the binding of peptide epitope specific constructs.

Thus, it was concluded that binding of the construct could be blocked by antibodies reacting with the binding site of HLA Class I (BB7.2 and W6/32) displayed by the construct (steric hindrance) and mouse anti human T-cell, CD8 Clone DK25 on the T-cells. It should be noted, however, that none of the inhibitory antibodies used in this study were added in saturating amounts.

EXAMPLE 8

Binding of the constructs of the invention: The effect of HLA Class I:dextran ratio during the process of ligation

In this experiment, the more optimal number of HLA Class I molecules per dextran carrier molecule required for maximal cell binding of MHC molecule constructs of the invention was analysed.

The following MHC molecule constructs of the invention were used:

MHC molecule construct 1, however, with different amounts of HLA A0201, cf. below,
MHC molecule construct 2, however, with different amounts of HLA A0201, cf. below,
MHC molecule construct 3, however, with different amounts of HLA A0201, cf. below.

Graded amounts of recombinant biotinylated HLA A0201 complexes displaying the MART-1 peptide analogue (ELAGIGILTV) were added to individual solutions of the constructs comprising different molecular sizes dextran carrier molecules, namely 150, 270 and 500 kDa dextran carrier molecules, respectively. More specifically, a 80 nM solution of 500 kDa dextran (conjugated with 14 SA, each labelled in average with 2 FITC) was incubated in

FACS-buffer (PBS, 10 mg/ml BSA, 0.2% azide) with 88- (7040 nM HLA A0201), 44- (3520 nM) and 14- (1121 nM) fold excess of mono-biotinylated HLA A0201 complexes, respectively. The reaction mixture was incubated 60 minutes at 22°C to obtain steady-state between the HLA A0201 and the SA molecules conjugated to the dextran. From the ratios of HLA A0201 and dextran (88, 44, 14 HLA molecules to one dextran molecules) ratios of HLA to SA during ligation corresponding to 6.5, 3.25 and 1, respectively, could be calculated. Due to the high affinity of SA and biotinylated MHC, it was expected that the ligation between HLA A0201 and SA per dextran resulted in fully saturated (88-fold excess of HLA), nearly saturated (44-fold excess of HLA) and partly saturated (14-fold excess HLA) MHC molecule constructs. Assuming 4 binding sites per SA, the molecule constructs are thus loaded with 54.4, 44.2 and 14.1 HLA A0201 molecules per dextran. The solutions were diluted 4-fold (final MHC molecule construct concentration of 20 nM) prior to usage for T-cell staining by flow cytometry following a standard protocol.

In a similar procedure, a 145 nM SA/dextran preparation (270 kDa, 7 SA per dextran, each SA labelled in average with 2 FITC) was ligated with biotinylated HLA A0201. The concentrations of HLA A0201 used for the ligation process were 48.5, 24.2 and 8.1 fold excess of the dextran concentration. The ratio of HLA:SA could be calculated to 7, 3.5 and 1.1, respectively. Assuming 4 binding sites per SA, the molecule constructs were thus loaded with 27.6, 24.2 and 7.6 HLA A0201 molecules per dextran. The solutions were diluted to 20 nM prior to T-cell staining following a standard procedure.

In a similar procedure, a 244 nM SA/dextran preparation (150 kDa, 4.4 SA per dextran, each SA labelled in average

with 2 FITC) was ligated with biotinylated HLA A0201. The concentrations of HLA A0201 used for the ligation process corresponded to 28.8 (7040 nM HLA A0201), 14.4 (3520 nM HLA A0201) and 7.2 (1760 nM HLA A0201) fold excess of the dextran concentration. The ratio of HLA:SA could be calculated to 6.5, 3.3 and 1.6, respectively. Assuming 4 binding sites per SA, the molecule constructs were thus loaded with 17.6, 14.5 and 7.0 HLA A0201 molecules per dextran. The solutions were diluted to 20 nM prior to T-cell staining following a standard procedure.

The T-cell clones were incubated for 60 minutes at 22°C prior to staining of the T-cell clone (5/127). The T-cell clones were incubated 60 minutes at room temperature with 20 nM solutions of the MHC molecule constructs loaded with different amounts of HLA A0201. The cells were subsequently washed once and analysed for cell bound construct by flow cytometry following standard flow cytometry procedure. All of the constructs bound as expected specifically to clone 5/127 (results shown Figure 30).

The construct comprising the larger (500 kDa) dextran carrier molecules was - in average - conjugated with 13,6 streptavidin molecules with a theoretical number of binding sites for biotinylated HLA molecules about 54 per dextran molecule (assuming 4 biotin binding sites per SA). In comparison, the construct comprising the 270 kDa and the 150 kDa dextran carrier molecules were conjugated with 6,9 and 4.4 streptavidin molecules per dextran, respectively, with a theoretical number of biotin binding sites corresponding to 42 and 17 biotinylated HLA Class I molecules, respectively.

As shown in Figure 30, the constructs comprising the 500 kDa dextran carrier molecule bound optimally to the T-

cells when loaded with a total of 44 HLA Class I molecules per dextran carrier molecule. In comparison, the constructs comprising the 270 and the 150 kDa dextran carrier molecules, respectively, bound optimally when loaded with totally 24.2 and 14.5 HLA Class I molecules per dextran (corresponding to about 4 bound HLA molecules per SA, respectively). The observed density of HLA Class I/dextran corresponded to well to 4 HLA Class I molecules/SA molecule conjugated to the dextran molecule. The observed reduction of T-cell staining using MHC molecule constructs generated in excess HLA molecules could be due to inhibition by unbound monomer HLA molecules. Thus, it was concluded that the MHC molecule constructs of the invention bound optimally to peptide specific T-cells when all available biotin binding sites of the carrier molecule were saturated during the process of ligation. Excess of unbound monomeric MHC molecule inhibited, however, the interaction between MHC molecule constructs and specific TCRs. Thus, the ligation process should, consequently, be performed with 1:1 ratio of MHC molecules to binding sites.

EXAMPLE 9

Binding of MHC molecule constructs and tetramers to small populations of T-cells

In this experiment, it was shown that binding of MHC molecule constructs of the invention provided improved detection of minor populations of specific T-cells as compared to tetramers.

The following MHC molecule construct of the invention was used:

MHC molecule construct 1.

The following tetramer was used:

Tetramer 1.

The tetramer was used for comparison.

The T-cell clones 5/127 recognising the peptide analogue from MART-1 and 5/130 recognising the peptide from gp100, respectively, were mixed in a ratio of 1:20 T-cell clone 5/127 to T-cell clone 5/130 and used for analysis of MHC molecule constructs of the invention and tetramers. The tetramer was used in 5-fold higher concentration to compensate for the lower binding avidity (cf. the findings of Example 3) as compared to the peptide-corresponding poly-ligand MHC molecule construct of the invention (cf. Figures 25 and 26).

The cell solution was incubated with 3 nM MHC molecule construct or 15 nM tetramer for 1 hour at room temperature. The cells were washed once and analysed by flow cytometry following standard flow cytometry procedures. As shown in Figure 31, both the construct of the invention and the tetramer stained about 5% of the cells corresponding to the MART-1 specific sub-population of 5/127 T-cell clones. The staining of cells by the construct of the invention provided, however, a clear distinction between positive and negative T-cells.

In comparison, the staining by the tetramer provided a less clear distinction between the two T-cell populations cf. Figure 31.

Consequently, it was concluded that the constructs of the invention provide better staining, thus, improved

capacity for detection by flow cytometry of minor T-cell populations in comparison to the prior art tetramers.

EXAMPLE 10

Pre-formed peptide empty MHC molecule constructs bind to specific T-cells after loading with appropriate peptides

The surprising and sensitive capacity of the MHC molecule constructs of the invention in the detection of small T-cell specificities (cf. Example 9) in mixed cell samples was further investigated using samples containing about 1% T-cell clone 5/127 ("high avidity T-cell clone", cf. Figure 25) and 1% 5/130 ("low avidity T-cell clone", cf. Figure 25. The percentage of 1 was chosen as a variety of studies have shown that a sub-population of proliferating T-cells frequently comprises approximately 1% of total number of T-cells in blood samples and within a range of 0.1 to 10% in immune responding patients.

The following peptide empty MHC molecule construct of the invention was generated from peptide empty HLA A0201 molecule construct of invention:

MHC molecule construct 7.

Also, MHC molecule construct 1 and MHC molecule construct 8 was used.

In this experiment a peptide specific peptide HLA A0201 molecule construct was further generated.

The following tetramer was produced from peptide empty HLA A0201 ligated to streptavidin labelled with PE:

Tetramer 6.

Also, tetramer 1 and tetramer 2 was used.

The peptide empty HLA molecule construct of the invention was formed by incubation of 60 pmol mono-biotinylated heavy chain (2 µl stock solution with 30 µM heavy chain molecule obtained as described in Example 1.C.) with 1 nmol β_2m in 198 µl dilution buffer (20 mM tris, pH 6.8, 150 mM NaCl) for 2 hours at 18°C. The formed dimer (approximately 270 nM) was stable in this buffer for several days when stored at 4°C. The peptide empty HLA molecule construct of the invention was formed by addition of streptavidin dextran carrier molecules (500 kDa, 13.6 SA/dextran, added to a final concentration of 10 nM) to 100 µl solution.

The peptide-displaying HLA molecule construct (construct 1) was formed by adding the MART-1 peptide analogue ELAGIGILTV to the solution of HLA dimers and dextran molecules to a final concentration of 10 µM and incubating over night at 18°C.

In a similar approach, MHC molecule construct 8 of the invention displaying a gp100 peptide was generated by addition of the peptide KTWGQYWOV.

The tetramers were generated in a similar approach except that PE-labelled SA was added sequentially to a final concentration of 70 nM, to ensure a ratio of 1:4 between SA and HLA molecules, prior to addition of the MART-1 peptide analogue ELAGIGILTV or gp100 peptide KTWGQYWOV. The tetramer was used for comparison.

Prior to the staining, the solutions with MHC molecule constructs of invention and the tetramers were diluted twice in FACS buffer described above containing 2 mg/ml

BSA and 0,2% azide. The concentrations of molecule of the constructs and the tetramers used for staining of T.cells were thus 5 and 35 nM, respectively.

The two T-cells clones 5/127 and 5/130, respectively, were mixed in ratios of 1:100, i.e. one cell sample contained about 1% 5/127 and 99% 5/130 T-cells and an other cell sample contained 1% 5/130 and 99% 5/127. The mixed cell solutions were tested for binding of construct of the invention or tetramers displaying peptides recognised by the 1% subpopulations of T-cells.

For flow cytometry, the cells (5×10^5) were centrifuged at 300g for 5 minutes, and re-suspended in 50 μl solution with MHC molecule construct of the invention or tetramer and incubated for 60 minutes at room temperature. Subsequently, the cells were washed once and immediately analysed by flow cytometry following standard procedures.

As shown in Figure 32, the MART-1 peptide (ELAGIGILTV)-displaying construct of the invention provided a clear distinction between positive T-cells (1,2% positively stained cells) whereas the construct displaying the gp100 peptide (KTWGQYWOV) stained about 0,4%. Although utilised in 7-fold higher concentration neither of the corresponding tetramers were able to stain the T-cells (data not shown).

Thus, it was concluded that it was indeed possible to generate peptide empty MHC molecule constructs of the invention. By subsequently loading with appropriate peptides, the resulting MHC molecule constructs were capable of staining minor populations of T-cells. In contrast to the tetramers, the MHC molecule constructs of invention were recognised by both low and high avidity T-cell clones using flow cytometry.

EXAMPLE 11

Binding of radio-labelled MHC molecule construct displaying the MART-1 peptide

In this experiment, the cell binding of a radio-labelled MHC molecule construct of invention was investigated. The molecule construct comprised as MHC molecule, HLA/peptide complexes folded in the presence of iodinated β_2m. The construct was prepared according to Example 1, however, with the folding taking place in the presence of iodinated β_2m.

The following MHC molecule construct of the invention was used:

a MHC molecule construct comprising the 500 kDa dextran carrier molecule having attached thereto 27.2 HLA A0201 heavy chain in complex with the MART-1 peptide analogue (ELAGIGILTV) and iodinated β_2m via 13.6 SA.

The β m were iodinated according to standard procedures and used for folding of fully biotinylated and active heavy chain as described above (cf. Example 1.C.). The de novo generated HLA A0201 complex comprising peptide, heavy chain and a radio-labelled β_2m molecule was purified from the excess of β_2m and peptide by G50 chromatography following standard protocol. The radio-activity was counted using a COBRA gamma counter prior to ligation of purified HLA A0201 complexes to SA conjugated dextrans.

Samples of the MART-1 or gp100 specific T-cell ($5x10^5$) clones 5/127 and 5/130 in 100 μl PBS with 1% BSA, were incubated with the radio-labelled MHC molecule construct

of the invention (100000 cpm/sample at 18°C for 1 hour with or without the variety of antibodies as described in Example 7. The cells were washed 5 times and transferred to fresh tubes prior to counting of cell bound radioactivity.

As shown in Figure 33, the 5/127 T-cells bound radio-labelled MHC molecule construct of the invention (MHC molecule construct 1), whereas the gp100 peptide specific T-cell clone 5/130 as expected did not bind.

Furthermore, it was observed that the antibodies BB7.2 and W6/32 but not BBM1 inhibited binding of the construct of the invention in agreement with the findings of Example 7.

Thus, it was concluded that the MHC molecule constructs of the invention comprising a radio-labelled β_2m were capable of binding to specific T-cells. Furthermore, the binding of this type of labelled MHC molecule construct was comparable to the binding of differently labelled MHC molecule constructs.

Another important feature was that labelling of β_2m represents in this context a versatile alternative to labelling of the heavy chain or the peptide, since the β_2m is a common subunit, which facilitates folding of a variety of different HLA molecules.

EXAMPLE 12

Staining of tumour specific T-cells

In this example, the ability of poly-ligand MHC molecule constructs to label specific T-cells in breast cancer lesions was tested. The test was performed on acetone-

fixed, frozen biopsies from human skin, lymph nodes and tumour lesions, respectively, mounted on slides.

The following MHC molecule constructs were used:

MHC molecule construct 8
MHC molecule construct 11.

In Figure 38, the staining of specific T-cells in HLA A2 positive biopsies taken from breast cancer lesions are shown. The staining was performed by poly-ligand MHC molecule constructs (500 kDa) displaying maximal amount of HLA-A0201 in association with the peptide analogue (SUR1M2)(LMLGEFLKL) from survivin, a recently identified tumour associated antigen.

The frozen tissue was sectioned and collected on glass slides (Superfrost Plus Gold Slides, Erie Scientific Co, Portsmouth, New Hampshire), air dried over-night and fixed in cold acetone for 5 minutes.

All the following procedure steps were performed at room temperature and in the dark. Between each step the slides was washed 3 times 10 minutes with a Phosphate Buffered Saline (PBS) buffer (pH 7.6).

The slides were firstly incubated (45 minutes) with (i) the primary antibody; anti-CD8 (anti-CD8 clone HIT8a, cat. No 550372, Pharmingen, San Diego, CA, USA, 1:100 dilution in PBS buffer), followed by (ii) Cy3-conjugated goat anti-mouse (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA, diluted 1:500 in PBS) for 45 minutes and finally (iii) incubated with the poly-ligand MHC molecule construct for 75 minutes (100 µml, 20 10-9 M construct in PBS).

Finally, the stained slides were mounted with coverglass in antifade solution (Vectashield, Vector labs, Burlingame, CA, USA) and kept in the refrigerator until analysis under the confocal microscope.

The entire population of cytotoxic TILS in tumour biopsies could be visualised by Cy3 conjugated anti-CD8 specific antibodies (Figure 25, left lanes) whereas the SUR1M2 specific T-cell clones could be visualised with FITC conjugated poly-ligand MHC molecule construct (right lane at top). Double staining of CD8 positive and peptide epitope specific T-cells were revealed in the merged pictures in the middle lanes (top). Another HLA A0201 binding peptide, the melanoma associated gp100 antigen displayed by the MHC molecule construct did not stain TILS in the examined breast cancer tissue (right lane, middle). In a second control, the SUR1M2 poly-ligand MHC molecule construct did not stain T-cells in breast cancer biopsies from A2 negative patients (right, bottom).

Thus, it was concluded that peptide specific poly-ligand MHC molecule bound specifically to a subtle target T-cell population and thus allowed *in situ* analyses of T-cell expression in biopsies from breast cancer patients.

EXAMPLE 13

<u>In situ staining of melanoma and lymph node tissues with SUR1M2 poly-ligand MHC molecule construct</u>

In this example the ability of poly-ligand MHC molecule constructs to stain specific T-cells in melanoma and lymph node tissue from HLA-A2 positive patient material was tested.

The following MHC molecule construct was used:

MHC molecule construct 11.

The experimental details of this Example are similar to those given in Example 12 except that tissue originated from a melanoma patient.

In Figure 39, the left lane Cy-3 staining of CD8+ T-cells in tissue samples from tumour (top) and lymph node (bottom) are shown. The right lane shows the localised staining by the FITC A2-SUR1M2 MHC molecule construct. Double staining of CD8 positive and SUR1M2 peptide specific T-cells are depicted in the middle lane showing the merged pictures.

In conclusion, it was shown that specific binding *in situ* of SUR1M2 peptide displaying poly-ligand MHC molecule construct to CD8+ T-cells in biopsies from melanoma lesions and lymph nodes could be detected.

EXAMPLE 14

In situ staining of CD8+ T-cells in melanoma tissue with MART-1 peptide displaying poly-ligand MHC molecule construct

In this example specific staining of melanoma tissue from a HLA-A 0201 positive patient by an A2-MART-1 MHC molecule construct was investigated.

The following MHC molecule construct was used:

MHC molecule construct 1.

The experimental details of this Example were similar to those given in Example 12, except that tissue was from a

melanoma patient and that the poly-ligand MHC molecule construct displayed the MART-1 peptide analogue (ELAGIGILTV).

The result of the experimental staining of a melanoma biopsy is given in Figure 40. The left picture shows the localisation of PE-stained CD-8 positive cells and the right picture the presence of FITC stained MART-1 specific T-cells. Double stained MART-1/CD-8 positive cells are seen in the merged middle picture.

In conclusion, using an approach similar to Example 12, it was shown that specific binding of poly-ligand MHC molecule construct displaying the MART-1 peptide analogue (ELAGIGILTV) to CD8 T-cells in a lesion from an HLA A0201 positive melanoma patient could be detected *in situ*.

EXAMPLE 15

<u>In situ staining of BV12 reactive and non-reactive T-cells in skin biopsies from injection sites using MART-1 and MAGE-3 peptide displaying poly-ligand MHC molecule constructs</u>

Attempts to develop curative immune therapy comprise strategies where soluble peptide candidates e.g. SUR1M2 and/or dendritic cells (DC) loaded with peptides or tumour lysates are injected in the patient. Whereas cellular immune responses are initiated by the interaction of T-cells and antigen presenting cells e.g. DC in secondary lymphoid organs, the therapeutic vaccinations may lead to another scenario namely local expansion and accumulation of antigen specific T-cells. Using poly-ligand MHC molecule constructs displaying tumour associated peptides, it was investigated whether

peptide specific T-cells are over-represented at the injection site.

The following MHC molecule constructs were used:

MHC molecule construct 1
MHC molecule construct 14.

Using an experimental approach similar to the one used in Example 12 in situ double staining analysis with Cy-3 labelled TCR VB12 antibody and FITC labelled poly-ligand MHC molecule constructs displaying the MART-1 or MAGE-3 peptide were performed on skin biopsies from injection sites. The TCR VB12 antibody only reacts with a subset of the T-cells as it is specific for T-cell receptors expressing the variable ß-chain family 12 region.

The results are shown in Figure 41. Left lanes reveals three distinct populations of T-cells. The populations include a BV12-/MART-1 reactive (A), BV12+/MART-1 reactive (B) as well as BV12+/MART-1 non-reactive cells (C). Thus, a non-specific interaction of MART-1/HLA A0201 poly-ligand MHC molecule construct with all members of the BV12 family could be excluded. Moreover, the MAGE-3 peptide recognising cells were found in small clusters, suggesting a local expansion of this T-cell specificity (D).

Thus in conclusion, using the poly-ligand MHC molecule constructs recognising specific T-cells it was possible to demonstrate the *in* situ presence of populations of specific T-cells at the injection site 48 hours after s.c. injection of tumour lysate pulsed DCs suggesting a local expansion of antigen specific T-cells.

EXAMPLE 16

In situ staining of CD8 reactive T-cells in skin biopsies from injection site using gp-100 and MAGE-3 peptide displaying poly-ligand MHC molecule constructs

In this experiment the accumulation of peptide antigen specific T-cells were further investigated using the same approach as in Example 15.

The following MHC molecule constructs were used:

MHC molecule construct 8
MHC molecule construct 14.

The experimental results are shown in Figure 42. Left and middle lanes show the staining with anti-CD8 antibodies and peptide specific poly-ligands, respectively. The merged pictures are shown in the right lane.

It was concluded that that immunisation of patients with DC pulsed with a gp100 peptide epitope led to infiltration of specific T-cells that recognised the peptide displayed by HLA A0201 poly-ligand MHC molecule construct (Figure 42B) but not a MAGE-3 epitope (Figure 42A).

EXAMPLE 17

Chromogen in situ staining of CD8+ T-cells in melanoma tissue with MART-1 peptide displaying poly-ligand MHC molecule construct

It was studied if specific binding of poly-ligand MHC molecule construct displaying the MART-1 peptide analogue (ELAGIGILTV) to CD8 T-cells in a lesion from an HLA A0201 positive melanoma patient, could be visualised by HRP-

mediated chromogen staining using two different peroxide blocking methods.

The following MHC molecule construct was used:

MHC molecule construct 13.

The frozen melanoma lesions were sectioned (5 μm) and collected on glass slides (Superfrost® Plus Gold Slides, Erie Scientific Co, Portsmouth, New Hampshire), air dried for 30 minutes and fixed in cold anhydrous reagent grade acetone (Aldrich, Milwaukee, WI, USA) for 5 minutes.

All the following procedure steps were performed at room temperature. Between each step the slides was washed batch vice 3 times 10 minutes with a PBS buffer (pH 7.6).

Endogenous peroxidase was blocked following two different reagent strategies:

A peroxide/methanol solution (50 ml 3% H_2O_2 plus 200 ml methanol) (Figure 43A) or peroxidase blocking solution (code S2023, DAKO A/S, Glostrup, Denmark) (Figure 43B).

After washing, the slides were incubated for 30 minutes with the indicated HLA-peptide dextran 270 HRP constructs (100 ml, 2.9 10-9 M in PBS).

After two washes, bound complexes were visualised using 3-amino-9-ethylcarbazol (AEC)-substrate (DAKO AEC Substrate System, DAKO A/S, Glostrup, Denmark). The reaction was terminated after 25 minutes.

The slides were counter stained with Mayer's hematoxylin (Code S330930, DAKO A/S, Glostrup, Denmark, 15 seconds) and washed in PBS buffer until slightly blue (about 30

seconds). Finally, the slides were coverslip mounted using Aquamont (DAKO Corporation, Carpenteria, CA, USA) and analysed using a bright field microscope (Zeiss) with photographic capacities.

In conclusion successful staining was achieved irrespective of the strategy used for blocking endogenous peroxidase.

EXAMPLE 18

T-cell activation induced by MHC molecule construct: the impact of co-stimulatory molecules

In this experiment, it was shown that activation of T-cell clones incubated with MHC construct is affected by the presence of co-stimulatory molecules attached to the MHC molecule construct, which bind to activating isoforms of NKRs.

The following MHC molecule constructs were used:

MHC molecule construct 15,
MHC molecule construct 16,
MHC molecule construct 17,
MHC molecule construct 18.

MHC molecule constructs 17 and 18 were used as controls.

Sub-optimal amounts of recombinant biotinylated HLA A0201 complexes displaying the MART-1 peptide anloug (ELAGIGILTV) or the gp100 peptide (KTWGQYWOV) were added to a solution of the construct comprising 500 kD dextran carrier molecules. More specifically, a 80 nM solution of dextran (conjugated with 13.6 SA, each labelled in average with 2 FITC) was incubated in PBS with 1121 nM

mono-biotinylated HLA A0201 complexes with or without 510 nM mono-biotinylated MIC A protein. It can be stipulated from Example 8 that the molecule constructs comprising only HLA complexes are loaded with 14.1 HLA A0201 molecules per dextran. The molecule constructs comprising HLA and MIC A protein are loaded with 14.1 HLA A0201 molecules per dextran and 7.1 MIC A molecules per dextran, respectively.

The MART-1 peptide/HLA A0201 specific T-cell clones 5/127 ($5x10^5$) was grown in media and incubated with 5 nM molecule constructs displaying either MART-1 or gp100 peptides with or without MIC A proteins. The cells were incubated for 24 and 48 hours, respectively, at 37 C, prior to measuring IFN-gamma in supernatants by ELISA following standard procedure.

As shown in Figure 44, the construct comprising HLA A0201 displaying MART-1 peptide combined with or without MIC A stimulated the 5/127 T-cell clone to IFN-gamma release after 24 hours, suggesting that both constructs were capable of binding and induce some signalling. In contrast, only the molecule construct comprising HLA and MIC A was capable of further stimulation as indicated by the increase amount of IFN-gamma. In comparison, the IFN-gamma release remained unchanged by stimulation of MHC molecule construct comprising only MART-1 peptide/HLA complexes. None of the MHC molecule constructs displaying gp100 peptide were capable of stimulation in this experiment (data not shown).

It was concluded that MHC molecule molecule constructs displaying appropriate peptide was able to stimulate T-cells upon binding to peptide specific TCR (cf. Figures 25B and 30). However, only molecule constructs comprising appropriate peptide-HLA complexes and MIC A protein

stimulated the T-cells upon prolonged incubation. This feature can be explained by induction of T-cell anergy when stimulated with molecule construct without co-stimulatory proteins. This construct was capable of initial stimulation followed by inactivation of the T-cells, which is characteristic feature of anergy. MHC molecule construct with HLA and MIC A was capable of continuous stimulation.

EXAMPLE 19

Preparation of carrier molecules having attached thereto a plurality of binding entities

Various carrier molecules (as exemplified by 150, 270 and 500 kDa dextrans, respectively) having attached thereto a plurality of binding entities (as exemplified by streptavidine (SA)) were prepared according to the procedure described below. MHC molecules and/or biologically active compounds can be attached subsequently. The theoretical number of coupling sites to each SA is 4, meaning that the loading capacity of each SA-dextran molecules is 22.4 (4×5.6), 41.2 (4×10.3) and 68 (4×17.0). Additionally MHC molecules/biologically compounds can be attached to the dextran molecule directly, thus, making the loading capacity even greater.

SA-Dextran (150, 270, 500 kDa)

Streptavidine (SA, Genzyme) was dialysed overnight (100 mg in 5 ml, against 1000 ml 0.10 M NaCl, 2-4°C, 10 kDa MwCO, changed three times). After UV absorbance measurement the concentration was calculated.

The SA solution was added to a solution of vinylsulfon-activated dextran (approximately 25% activated) of 150, 270 or 500 kDa (in total 1.6 mg vinylsulfon dextran/ml,

7.7 mg SA/ml, 0.1 M NaCl, 25 mM carbonate buffer, pH 8.5) and stirred at 30°C for 18 hours. Any remaining reactive groups were quenched by addition of 1/10 volume reaction mixture of an ethanol amine-containing buffer (110 mM ethanolamine, 50 mM HEPES, 0.1 M NaCl, pH 7.0) and stirred for 30 minutes at 30°C. The so obtained polymeric molecules (SA-dextran) was purified from unbound SA by gel filtration (FPLC, Pharmacia, S-200, 0.1 M HEPES, 0.1 M NaCl, pH 7.2).

The degree of SA incorporation per dextran molecule were calculated from the UV absorbance at 278 nm. The incorporation of SA was in average 5.6 (for the 150 kDa dextran), 10.3 (for the 270 kDa dextran) and 17.0 (for the 500 kDa dextran), respectively. The molecules were concentrated to the equivalent of 3.0 mg SA/mL using a Millipore filter centrifuge device.

EXAMPLE 20

Preparation of carrier molecules having attached thereto a plurality of labelled binding entities

By the procedures described below, various carrier molecules (as exemplified by 150, 270 and 500 kDa dextrans, respectively) having attached thereto a plurality of binding entities (as exemplified by streptavidine (SA)) labelled with a plurality of labelling compounds (as exemplified by Alexa 647) were prepared. MHC molecules and/or biologically active compounds can be attached subsequently. The theoretical number of coupling sites to each SA is 4, meaning that the loading capacity of each SA-dextran molecules is 22.4 (4×5.6), 41.2 (4×10.3) and 68 (4×17.0). Additionally MHC molecules/biologically compounds can be attached to the

dextran molecule directly, thus, making the loading capacity even greater.

Alexa 647 labelled SA-Dextran (150, 270, 500 kDa)

The SA dextran molecules obtained from Example 19 were labelled with Alexa 647 according to the general guidelines given by the manufacturer of the Alexa Fluor 647 Protein labelling Kit (Molecular Probes, product number A-20173). The reaction conditions were 1 vial Alexa 647, 2.0 mg SA/mL, 0.10 M NaCl, 50 mM carbonate, pH 8.0, 0.500 mL in total volume, 30°C, in the dark for one hour). Any remaining reactive groups were quenched by addition of 0.050 mL volume reaction mixture to an ethanol amine containing buffer (110 mM ethanol amine, 50 mM HEPES, 0.1 M NaCl, pH 7.0), and stirred for 30 minutes at 30°C. The so obtained fluorescently labelled polymeric molecules were purified from unbound dye by dialysis (against 1000 ml 0.10 M NaCl, 2-4°C, 10 kDa MwCO, changed three times), 0.1 M HEPES, 0.1 M NaCl, pH 7.2).

The degree of SA incorporation per dextran molecule, and Alexa 647 incorporation per SA, as well as molecule concentration were calculated from the UV absorbance at 278 and 650 nm. The molecules were added sodium azide to 15 mM as a preservative. The results are shown below.

Dextran carier molecule	SA per dextran (in average	Alexa 647 per SA (in average)	Concentration of dextran (mole/l)
150	5.6	2.7	60×10^{-8}
270	10.3	2.6	50×10^{-8}
500	17.0	2.7	20×10^{-8}

EXAMPLE 21

Preparation of carrier molecules having attached thereto a plurality of binding entities

By the procedures described below, various carrier molecules (as exemplified by 150, 270 and 500 kDa dextrans, respectively) having attached thereto a plurality of binding entities (as exemplified by rabbit-anti-biotin antibody) were prepared. MHC molecules and/or biologically active compounds can be attached subsequently as desired.

Rabbit-anti-biotin dextran (150, 270, 500 kDa)

Rabbit-anti-biotin antibody (affinity purified, Fab2, approximately 100 kDa, DAKO code number DM0069) was dialysed overnight (100 mg antibody in 5 ml, against 1000 ml 0.10 M NaCl, 2-4°C, 10 kDa MwCO, changed three times). After UV absorbance measurement, the concentration was calculated. The antibody solution was added to a solution of vinylsulfon-activated dextran (approximately 25% activated) of 150, 270 or 500 kDa (in total 0.680 mL, 1.07 mg vinylsulfon dextran/ml, 15.25 mg antibody/ml, 0.1 M NaCl, 25 mM carbonate buffer, pH 8.5), respectively, and stirred at 30°C for 18 hours. Any remaining reactive groups were quenched by addition of 1/10 volume reaction mixture of an ethanol amine-containing buffer (110 mM ethanolamine, 50 mM HEPES, 0.1 M NaCl, pH 7.0) and

stirred for 30 minutes at 30°C. The obtained polymeric molecules were purified from unbound antibody by gel filtration (FPLC, Pharmacia, S-200, 0.1 M HEPES, 0.1 M NaCl, pH 7.2).

The degree of antibody incorporation per dextran was calculated from the UV absorbance at 278 nm. The number of antibodies per dextran was in average 8.4 (for the 150 kDa dextran), 19.5 (for the 270 kDa dextran) and 34.4 (for the 500 kDa dextran). The molecules were concentrated to the equivalent of 3.9, 3.4 and 3.4 mg antibody/mL, respectively, using a Millipore filter centrifuge device.

In another preperation using the same conditions, the incorporation of antibodies per dextran was 7.2 (for the 150 kDa dextran) and 11.2 (for the 500 kDa dextran). These molecules were concentrated to the equivalent of 3.5 mg antibody/mL respectively using a Millipore filter centrifuge device.

EXAMPLE 22

Preparation of carrier molecules having attached thereto a plurality of labelled binding entities

By the procedures described below, various carrier molecules (as exemplified by 150 and 270 kDa dextrans, respectively) having attached thereto a plurality of binding entities (as exemplified by rabbit-anti-biotin antibody) labelled with a plurality of labelling compounds (as exemplified by Alexa 532 or Alexa 647) were prepared. MHC molecules and/or biologically active compounds can be attached subsequently as desired.

Preparation of Alexa 532 or 647 labelled rabbit-anti-biotin dextran

The rabbit-anti-biotin dextran molecules obtained in Example 21 were labelled with Alexa 532 or Alexa 647 according to the general guidelines given by the manufacturer of the Alexa Fluor 532 Protein labelling Kit (Molecular Probes, product number A-10236) or Fluor 647 Protein labelling Kit (Molecular Probes, product number A-20173). The reaction conditions were 1 vial Alexa dye, equivalent of 2.0 mg antibody/mL, 0.10 M NaCl, 50 mM carbonate, pH 8.0, 0.500 mL in total volume, 30°C, in the dark for one hour). Any remaining reactive groups were quenched by addition of 0.050 mL volume reaction mixture to an ethanol amine containing buffer (110 mM ethanol amine, 50 mM HEPES, 0.1 M NaCl, pH 7.0) and stirred for 30 minutes at 30°C. The four different fluorescently labelled polymeric molecules were purified from unbound dye by dialysis (against 1000 ml 0.10 M NaCl, 2-4°C, in the dark, 10 kDa MwCO, changed three times), 0.1 M HEPES, 0.1 M NaCl, pH 7.2).

The degree of Alexa 532 incorporation per antibody, and antibody incorporation per dextran, as wells as concentration were calculated from the UV absorbance at 278 and 530 nm. The molecules were added sodium azide to 15 mM as a preservative.

The degree of Alexa 647 incorporation per antibody, and antibody incorporation per dextran, as well as concentration were calculated from the UV absorbance at 278 and 650 nm. The molecules were added sodium azide to 15 mM as a preservative.

Dextran carrier molecule	Antibody per dextran (in average)	Alexa 532 per antibody (in average)	Concentration dextran (mole/l)
150	8.4	3.0	165.5×10^{-8}
270	19.5	2.9	69.4×10^{-8}

Dextran carrier molecule	Antibody per dextran (in average)	Alexa 647 per antibody (in average)	Concentration dextran (mole/l)
150	7.21	2.7	150×10^{-8}
270	11.2	2.6	65×10^{-8}

EXAMPLE 23

Preparation of carrier molecules having attached thereto a plurality of labelled binding entities

By the procedures described below, various carrier molecules (as exemplified by 150 and 270 kDa dextrans, respectively) having attached thereto a plurality of binding entities (as exemplified by rabbit-anti-biotin antibody) labelled with a plurality of labelling compounds (as exemplified by FITC) were prepared. MHC molecules and/or biologically active compounds can be attached subsequently as desired.

Preparation of FITC labelled Rabbit-anti-biotin dextrans

The rabbit-anti-biotin dextrans from Example 21 (150 kDa and 270 kDa dextrans) were used for FITC labelling). The FITC vial (FITC (fluorescein isothiocyanate), Molecular Probes, product number F-1906) storred in the freezer was allowed to stand at room temperature for one hour before being opened. A FITC solution (10.1 mg/ml NMP) was added to stirred mixtures of rabbit-anti-biotin dextran molecules (in total 0.750 ml, molecule concentration

equivalent to 1.5 mg antibody/ml, 0.0487 mg FITC/ml, equivalent to approximately 8.3 FITC per antibody, 0.1 M NaCl, 200 mM carbonate buffer, pH 8.5, 30°C, one hour in the dark). Any remaining reactive groups were quenched by addition of 1/10 volume reaction mixture of an ethanol amine-containing buffer (110 mM ethanol amine, 50 mM HEPES, 0.1 M NaCl, pH 7.0) and stirred for 30 minutes at 30°C. The two different FITC labelled rabbit-anti-biotin polymeric molecules were purified from unbound fluorescein by dialysis in a float-a-lyzer(against 500 ml 0.10 M NaCl, 2-4°C, in the dark, 10 kDa MwCO, changed three times), 0.1 M HEPES, 0.1 M NaCl, pH 7.2).

The degree of fluorescein incorporation per antibody, and antibody incorporation per dextran was calculated from the UV absorbance at 278 and 498 nm. The molecules were added sodium azide to 15 mM as a preservative.

Dextran carier molecule	Antibody per dextran (in average	Fluorescein per antibody (in average)	Concentration of dextran (mole/l)
150	8.4	1.7	125.2×10^{-8}
270	19.5	1.8	41.6×10^{-8}

EXAMPLE 24

Isolation of CTLs using the MHC molecules in an immunomagnetic separation procedure

In this experiment, it was shown that antigen reactive cytotoxic T lymphocytes (CTL) could be isolated from an HLA-A0201 positive patient lymph node sample by the use of MHC molecules immobilised on magnetic beads.

Single cell suspensions from melanoma infiltrated lymph node biopsy material were obtained after homogenisation

and centrifugation to remove cellular debris. The cell isolation was performed by using magnetic beads (Dynabeads®, with streptavidin) coated with biotinylated MHC molecules displaying HLA A0201 in association with the peptide analogue (SUR1/M2) (LMLGEFLKL) from survivin, a recently identified tumour associated antigen. The magnetic beads with immobilised MHC molecule were added to the cell suspension and incubated for 30 minutes at 30°C to allow the beads to bind to the cells. After binding, rosetted cells were isolated by using a magnet. In Figure 45, the results are shown. In Figure 45A, the bright field microscopy picture of the so isolated survivin reactive CTLs bound to the MHC molecule construct-coated beads are shown.

In the same experiment, magnetic beads coated with a biotinylated recombinant HLA A0201/influenza peptide was used as negative control. As shown in Figure 45B, magnetic beads coated with the HLA A0201/influenza peptide complexes did not bind to CTL cells from the melanoma infiltrated lymph node biopsy material.

Thus, it is expected that the high avidity of the MHC molecule constructs of the invention will result in even better specific binding to cells of interest, and accordingly that such cells are obtainable using the MHC molecule constructs of the invention.

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CLAIMS

1. A MHC molecule construct comprising

a carrier molecule having attached thereto one or more MHC molecules, said MHC molecules being attached to the carrier molecule either directly or via one or more binding entities.

2. The MHC molecule construct according to claim 1, wherein the MHC molecule is a vertebrate MHC molecule such as a human, a murine, a rat, a porcine, a bovine or an avian molecule.

3. The MHC molecule construct according to claim 1 or 2, wherein the MHC molecule is a human MHC molecule.

4. The MHC molecule construct according to any one of claims 1-3, wherein the MHC molecule is

a MHC Class I molecule selected from the group consisting of a heavy chain, a heavy chain combined with a β_2m, a heavy chain combined with a peptide, and a heavy chain/β_2m dimer with a peptide;

or a MHC Class II molecule selected from the group consisting of an α/β dimer, an α/β dimer with a peptide, α/β dimer combined through an affinity tag and a α/β dimer combined through an affinity tag with a peptide;

or a MHC Class I like molecule or MHC Class II like molecule.

5. The MHC molecule construct according to any one of claims 1-4, wherein the MHC molecule is a peptide free MHC molecule.

6. The MHC molecule construct according to any one of claims 1-5, wherein at least two of the MHC molecules are different.

7. The MHC molecule construct according to any one of claims 1-5, wherein the MHC molecules are the same.

8. The MHC molecule construct according to any one of claims 1-7, wherein at least two of the peptides harboured by the MHC molecules are different.

9. The MHC molecule construct according to any one of claims 1-7, wherein the peptides harboured by the MHC molecules are the same.

10. The MHC molecule construct according to any one of claims 1-9, wherein the MHC molecules are attached to the carrier molecule directly.

11. The MHC molecule construct according to any one of claims 1-9, wherein the MHC molecules are attached to the carrier molecule via one or more binding entities.

12. The MHC molecule construct according to claim 11, wherein each binding entity has attached thereto from 1 to 10 MHC molecules.

13. The MHC molecule construct according to claim 11, wherein each binding entity has attached thereto from 1 to 8 MHC molecules.

14. The MHC molecule construct according to claim 11, wherein each binding entity has attached thereto from 1 to 6 MHC molecules.

15. The MHC molecule construct according to claim 11, wherein each binding entity has attached thereto from 1 to 4 MHC molecules.

16. The MHC molecule construct according to claim 11, wherein each binding entity has attached thereto from 1 to 3 MHC molecules.

17. The MHC molecule construct according to claim 11, wherein each binding entity has attached thereto 1 or 2 MHC molecules.

18. The MHC molecule construct according to any one of claims 1-17, wherein the total number of MHC molecules of the construct is from 1 to 100.

19. The MHC molecule construct according to any one of claims 1-17, wherein the total number of MHC molecules of the construct is from 1 to 50.

20. The MHC molecule construct according to any one of claims 1-17, wherein the total number of MHC molecules of the construct is from 1 to 25.

21. The MHC molecule construct according to claim 1, wherein the binding entity is selected from streptavidin (SA) and avidin and derivatives thereof, biotin, immunoglobulins, antibodies (monoclonal, polyclonal, and recombinant), antibody fragments and derivatives thereof, leucine zipper domain of AP-1 (jun and fos), hexa-his (metal chelate moiety), hexa-hat GST (glutathione S-tranferase) glutathione affinity, Calmodulin-binding peptide (CBP), Strep-tag, Cellulose Binding Domain, Maltose Binding Protein, S-Peptide Tag, Chitin Binding Tag, Immuno-reactive Epitopes, Epitope Tags, E2Tag, HA Epitope Tag, Myc Epitope, FLAG Epitope, AU1 and AU5

Epitopes, Glu-Glu Epitope, KT3 Epitope, IRS Epitope, Btag Epitope, Protein Kinase-C Epitope, VSV Epitope, lectins that mediate binding to a diversity of compounds, including carbohydrates, lipids and proteins, e.g. Con A (*Canavalia ensiformis*) or WGA (wheat germ agglutinin) and tetranectin or Protein A or G (antibody affinity).

22. The MHC molecule construct according to any one of claims 1-21, further comprising one or more biologically active molecules.

23. The MHC molecule construct according to clam 22, wherein the biologically active molecules is selected from proteins, co-stimulatory molecules, cell modulating molecules, receptors, accessory molecules, adhesion molecules, natural ligands, and toxic molecules, and antibodies and recombinant binding molecules thereto, and combinations thereof.

24. The MHC molecule construct according to claims 22 or 23, wherein the biologically active molecule is attached to the carrier molecule either directly or via one or more of the binding entities.

25. The MHC molecule construct according to any one of claims 22-24, wherein the biologically active molecule is selected from

proteins such as MHC Class I-like proteins like MIC A, MIC B, CD1d, HLA E, HLA F, HLA G, HLA H, ULBP-1, ULBP-2, and ULBP-3,

co-stimulatory molecules such as CD2, CD3, CD4, CD5, CD8, CD9, CD27, CD28, CD30, CD69, CD134 (OX40), CD137 (4-1BB), CD147, CDw150 (SLAM), CD152 (CTLA-4), CD153 (CD30L), CD40L (CD154), NKG2D, ICOS, HVEM, HLA Class II, PD-1, Fas

(CD95), FasL expressed on T and/or NK cells, CD40, CD48, CD58, CD70, CD72, B7.1 (CD80), B7.2 (CD86), B7RP-1, B7-H3, PD-L1, PD-L2, CD134L, CD137L, ICOSL, LIGHT expressed on APC and/or tumour cells,

cell modulating molecules such as CD16, NKp30, NKp44, NKp46, NKp80, 2B4, KIR, LIR, CD94/NKG2A, CD94/NKG2C expressed on NK cells, IFN-alpha, IFN-beta, IFN-gamma, IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, CSFs (colony-stimulating factors), vitamin D3, IL-2 toxins, cyclosporin, FK-506, rapamycin, TGF-beta, clotrimazole, nitrendipine, and charybdotoxin,

accessory molecules such as LFA-1, CD11a/18, CD54 (ICAM-1), CD106 (VCAM), and CD49a,b,c,d,e,f/CD29 (VLA-4),

adhesion molecules such as ICAM-1, ICAM-2, GlyCAM-1, CD34, anti-LFA-1, anti-CD44, anti-beta7, chemokines, CXCR4, CCR5, anti-selectin L, anti-selectin E, and anti-selectin P,

toxic molecules such as cyclophosphamide, methrotrexate, Azathioprine, mizoribine, 15-deoxuspergualin, neomycin, staurosporine, genestein, herbimycin A, Pseudomonas exotoxin A, saporin, Rituxan, Ricin, gemtuzumab ozogamicin, Shiga toxin, heavy metals like inorganic and organic mercurials, and FN18-CRM9, radioisotopes such as incorporated isotopes of iodide, cobalt, selenium, tritium, and phosphor, and haptens such as DNP, and digoxiginin,

and antibodies thereto, or antibody derivatives or fragments thereof, and combinations thereof.

26. The MHC molecule construct according to any of claims 1-25 further comprising one or more labelling compounds.

27. The MHC molecule construct according to claim 26, wherein one or more labelling compounds are attached to the carrier molecule.

28. The MHC molecule construct according to claim 26, wherein one or more labelling compounds are attached to one or more of the binding entities.

29. The MHC molecule construct according to claim 26, wherein one or more labelling compounds are attached to one or more of the MHC molecules.

30. The MHC molecule construct according to claim 26, wherein one or more labelling compounds are attached to the carrier molecule and/or one or more of the binding entities and/or one or more of the MHC molecules.

31. The MHC molecule construct according to any one of claims 26-30, wherein the labelling compound is directly or indirectly detectable.

32. The MHC molecule construct according to any of claims 26-31, wherein the labelling compound is a fluorescent label, an enzyme label, a radioisotope, a chemiluminescent label, a bioluminescent label, a polymer, a metal particle, a hapten, an antibody, or a dye.

33. The MHC molecule construct according to any one of claims 26-32, wherein the labelling compound

is selected from fluorescent labels such as 5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate (F)TC), rhodamine, tetrameth-

ylrhodamine, and dyes such as Cy2, Cy3, and Cy5, optionally substituted coumarin including AMCA, PerCP, phycobiliproteins including R-phycoerythrin (RPE) and allophycoerythrin (APC), Texas Red, Princeston Red, Green fluorescent protein (GFP) and analogues thereof, and conjugates of R-phycoerythrin or allophycoerythrin and e.g. Cy5 or Texas Red, and inorganic fluorescent labels based on semiconductor nanocrystals (like quantum dot and Qdot™ nanocrystals), and time-resolved fluorescent labels based on lanthanides like Eu3+ and Sm3+,

from haptens such as DNP, biotin, and digoxiginin, or

is selected from enzymatic labels such as horse radish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase (GAL), glucose-6-phosphate dehydrogenase, beta-N-acetylglucosaminidase, ß-glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO), or

is selected from luminiscence labels such as luminol, isoluminol, acridinium esters, 1,2-dioxetanes and pyridopyridazines, or

is selected from radioactivity labels such as incorporated isotopes of iodide, cobalt, selenium, tritium, and phosphor.

34. The MHC molecule construct according to any one of claims 1-33, wherein the carrier molecule is selected from

polysaccharides including dextrans, carboxy methyl dextran, dextran polyaldehyde, carboxymethyl dextran lactone, and cyclodextrins,

pullulans, schizophyllan, scleroglucan, xanthan, gellan, O-ethylamino guaran, chitins and chitosans indlucing 6-O-carboxymethyl chitin and N-carboxymethyl chitosan,

derivatised cellolosics including carboxymethyl cellulose, carboxymethyl hydroxyethyl cellulose, hydroxy-ethyl cellulose, 6-amino-6-deoxy cellulose and O-ethyl-amine cellulose,

hydroxylated starch, hydroxypropyl starch, hydroxyethyl starch, carrageenans, alginates, and agarose,

synthetic polysaccharides including ficoll and carboxy-methylated ficoll,

vinyl polymers including poly(acrylic acid), poly(acryl amides), poly(acrylic esters), poly(2-hydroxy ethyl meth-acrylate), poly(methyl methacrylate), poly(maleic acid), poly(maleic anhydride), , poly(acrylamide), poly(ethyl-co-vinyl acetate), poly(methacrylic acid), poly(vinyl-alcohol), poly(vinyl alcohol-co-vinyl chloroacetate), aminated poly(vinyl alcohol), and co block polymers thereof,

poly ethylene glycol (PEG) or polypropylene glycol or poly(ethylene oxide-co-propylene oxides) containing polymer backbones including linear, comb-shaped or StarBurst™ dendrimers,

poly amino acids including polylysines, polyglutamic acid, polyurethanes, poly(ethylene imines), pluriol.

proteins including albumins, immunoglobulins, and virus-like proteins (VLP), and

polynucleotides, DNA, PNA, LNA, oligonucleotides and oligonucleotide dendrimer constructs.

35. The MHC molecule construct according to any one of claims 1-34, wherein the carrier molecule is a soluble carrier molecule.

36. The MHC molecule construct according to any one of claims 1-35 in soluble form.

37. The MHC molecule construct according to any one of claims 1-36 immobilised onto a solid or semi-solid support.

38. The MHC molecule construct according to claim 37, immobilised directly to the solid or semi-solid support.

39. The MHC molecule construct according to claim 37, immobilised to the solid or semi-solid support via a linker, a spacer, or an antibody, an antibody derivative or a fragment therof.

40. The MHC molecule construct according to any one of claims 37-39, wherein the support is selected from particles, beads, biodegradable particles, sheets, gels, filters, membranes (e. g. nylon membranes), fibres, capillaries, needles, microtitre strips, tubes, plates or wells, combs, pipette tips, micro arrays, and chips.

41. The MHC molecule construct according to claim 40, wherein the support is selected from beads and particles.

42. The MHC molecule construct according to claim 41, wherein the beads and particles are polymeric beads, polymeric particles, magnetic beads, magnetic particles, supermagnetic beads, or supermagnetic particles.

43. The MHC molecule construct according to any one of claims 1-42 for use in a flow cytometric method.

44. The MHC molecule construct according to any one of claims 1-42 for use in a histological method.

45. The MHC molecule construct according to any one of claims 1-42 for use in a cytological method.

46. A method for detecting the presence of MHC recognising cells in a sample comprising the steps of

(a) providing a sample suspected of comprising MHC recognising cells,
(b) contacting the sample with a MHC molecule construct according to claims 1-42, and
(c) determining any binding of the MHC molecule construct, which binding indicates the presence of MHC recognising cells.

47. A method for monitoring MHC recognising cells comprising the steps of

(a) providing a sample suspected of comprising MHC recognising cells,
(b) contacting the sample with a MHC molecule construct according to claims 1-42, and
(c) determining any binding of the MHC molecule construct, thereby monitoring MHC recognising cells.

48. A method for establishing a prognosis of a disease involving MHC recognising cells comprising the steps of

(a) providing a sample suspected of comprising MHC recognising cells,

(b) contacting the sample with a MHC molecule construct according to claims 1-42, and
(c) determining any binding of the MHC molecule construct, thereby establishing a prognosis of a disease involving MHC recognising cells.

49. A method for determining the status of a disease involving MHC recognising cells comprising the steps of

(a) providing a sample suspected of comprising MHC recognising cells,
(b) contacting the sample with a MHC molecule construct according to claims 1-42, and
(c) determining any binding of the MHC molecule construct, thereby determining the status of a disease involving MHC recognising cells.

50. A method for diagnosing a disease involving MHC recognising cells comprising the steps of

(a) providing a sample suspected of comprising MHC recognising cells,
(b) contacting the sample with a MHC molecule construct according to claims 1-42, and
(c) determining any binding of the MHC molecule construct, thereby diagnosing a disease involving MHC recognising cells.

51. A method for determining the effectiveness of a medicament against a disease involving MHC recognising cells comprising the steps of

(a) providing a sample from a subject receiving treatment with a medicament,
(b) contacting the sample with a MHC molecule construct according to claims 1-42, and

(c) determining any binding of the MHC molecule construct, thereby determining the effectiveness of the medicament.

52. The method according to any one of claims 46-51, wherein the MHC recognising cells are involved in a disease of inflammatory, auto-immune, allergic, viral, cancerous, infectious, allo- or xenogene (graft versus host and host versus graft) origin.

53. The method according to claim 52, wherein the disease is a chronic inflammatory bowel disease such as Crohn's disease or ulcerative colitis, sclerosis, type I diabetes, rheumatoid arthritis, psoriasis, atopic dermatitis, asthma, malignant melanoma, renal carcinoma, breast cancer, lung cancer, cancer of the uterus, cervical cancer, prostatic cancer, brain cancer, head and neck cancer, leukaemia, cutaneous lymphoma, hepatic carcinoma, colorectal cancer, bladder cancer, rejection-related disease, Graft-versus-host-related disease, or a viral disease associated with hepatitis, AIDS, measles, pox, chicken pox, rubella or herpes.

54. The method according to any one of claims 46-53, wherein the MHC recognising cells selected from subpopulations of CD3+ T-cells, gamma,delta T-cells, alpha,beta T-cells, CD4+ T-cells, T helper cels, CD8+ T-cells, Suppressor T-cells, CD8+ cytotoxic T-cells, CTLs, NK cells, NKT cells, LAK cells, and MAK.

55. The method according to any one of claims 46-51, wherein the sample is selected from histological material, cytological material, primary tumours, secondary organ metastasis, fine needle aspirates, spleen tissue, bone marrow specimens, cell smears, exfoliative cytological specimens, touch preparations, oral swabs,

laryngeal swabs, vaginal swabs, bronchial lavage, gastric lavage, from the umbilical cord, and from body fluids such as blood (e.g. from a peripheral blood mononuclear cell (PBMC) population isolated from blood or from other blood-derived preparations such as leukopheresis products), from sputum samples, expectorates, and bronchial aspirates.

56. The method according to any one of claims 46-55, wherein the determination of the binding is carried out by inspection in a microscope, by light, by fluorescence, by electron transmission, or by flow cytometry.

57. The method according to any one of claims 46-56, wherein the sample is mounted on a support.

58. The method according to claim 57, wherein the support is a solid or semi-solid support.

59. The method according to claim 57 or 58, wherein the support is selected from glass slides, microtiter plates having one or more wells, beads, particles, membranes, filters, filter membranes, polymer slides, polymer membranes, chamber slides, dishes, and petridishes.

60. A composition comprising a MHC molecule construct according to any one of claims 1-42 in a solubilising medium.

61. The composition according to claim 60, wherein the MHC molecule construct comprises peptide filled MHC molecules.

62. The composition according to claim 60, wherein the MHC molecule construct comprises peptide free MHC molecules.

63. The composition according to claim 62, wherein peptides to fill the peptide free MHC molecules, and the MHC molecule construct comprising peptide free molecules are provided separately.

64. A composition comprising a MHC molecule construct according to any one of claims 1-42, wherein the MHC molecule construct is immobilised onto a solid or semi-solid support.

65. The composition according to claim 64, wherein the support is selected from glass slides, microtiter plates having one or more wells, beads, particles, membranes, filters, filter membranes, polymer slides, polymer membranes, chamber slides, dishes, and petridishes.

66. The composition according to claim 64 or 65, wherein the beads and particles are polymeric beads, polymeric particles, magnetic beads, magnetic particles, supermagnetic beads, or supermagnetic particles.

67. The composition according to claim 64, wherein the MHC molecule construct comprises peptide filled MHC molecules.

68. The composition according to claim 64, wherein the MHC molecule construct comprises peptide free MHC molecules.

69. The composition according to claim 68, wherein peptides to fill the peptide free MHC molecules, and the MHC molecule construct comprising peptide free molecules are provided separately.

70. Use of a MHC molecule construct according to any one of claims 1-42 as a detection system.

71. Use of a MHC molecule construct according to any one of claims 1-42 for diagnosing a disease involving MHC recognising cells.

72. Use of a MHC molecule construct according to any one of claims 1-42 for monitoring a disease involving MHC recognising cells.

73. Use of a MHC molecule construct according to any one of claims 1-42 for establishing a prognosis for a disease involving MHC recognising cells.

74. Use of a MHC molecule construct according to any one of claims 1-42 for determining the status of a disease involving MHC recognising cells.

75. Use of a MHC molecule construct according to any one of claims 1-42 for determining the effectiveness of a medicament against a disease involving MHC recognising cells.

76. Use according to any one of claims 71, wherein the the MHC recognising cells are involved in a disease of inflammatory, auto-immune, allergic, viral, cancerous, infectious, allo- or xenogene (graft-versus-host and host-versus-graft) origin.

77. Use according to claim 76, wherein the disease is a chronic inflammatory bowel disease such as Crohn's disease or ulcerative colitis, sclerosis, type I diabetes, rheumatoid arthritis, psoriasis, atopic dermatitis, asthma, malignant melanoma, renal carcinoma, breast cancer, lung cancer, cancer of the uterus,

cervical cancer, prostatic cancer, brain cancer, head and neck cancer, leukaemia, cutaneous lymphoma, hepatic carcinoma, colorectal cancer, bladder cancer, rejection-related disease, Graft-versus-host-related disease, or a viral disease associated with hepatitis, AIDS, measles, pox, chicken pox, rubella or herpes.

78. Use according to any one of claims 70-77, wherein the MHC recognising cells are selected from subpopulations of CD3+ T-cells, gamma,delta T-cells, alpha,beta T-cells, CD4+ T-cells, T helper cels, CD8+ T-cells, Suppressor T-cells, CD8+ cytotoxic T-cells, CTLs, NK cells, NKT cells, LAK cells, and MAK.

79. The MHC molecule construct according to any one of claims 1-42 for use as a therapeutic composition.

80. The MHC molecule construct according to any one of claims 1-42 for use in in vivo therapy.

81. The MHC molecule construct according to any one of claims 1-42 for use in ex vivo therapy.

82. A therapeutic composition comprising as active ingredient a MHC molecule construct as defined in any one of claims 1-42.

83. The therapeutic composition according to claim 82, wherein the MHC molecule construct is immobilised to a biodegradable solid or semi-solid support.

84. The therapeutic composition according to claim 82 or 83, wherein the MHC molecule construct comprises

a carrier molecule having attached thereto one or more MHC molecules, said MHC molecules being attached to the

carrier molecule either directly or via one or more binding entities.

85. The therapeutic composition according to claim 82 or 83, wherein the MHC molecule is a vertebrate MHC molecule such as a human, a murine, a rat, a porcine, a bovine or an avian molecule.

86. The therapeutic composition according to any one of claims 82-85, wherein the MHC molecule is a human MHC molecule.

87. The therapeutic composition according to any one of claims 82-86, wherein the MHC molecule is

a MHC Class I molecule selected from the group consisting of a heavy chain, a heavy chain combined with a β_2m, a heavy chain combined with a peptide, and a heavy chain/β_2m dimer with a peptide;

or a MHC Class II molecule selected from the group consisting of an α/β dimer, an α/β dimer with a peptide, α/β dimer combined through an affinity tag and a α/β dimer combined through an affinity tag with a peptide

or a MHC Class I like molecule or a MHC Class II like molecule.

88. The therapeutic composition according to any one of claims 82-87, wherein the MHC molecule is a peptide free MHC molecule.

89. The therapeutic composition according to any one of claims 82-88, wherein at least two of the MHC molecules are different.

90. The therapeutic composition according to any one of claims 82-88, wherein the MHC molecules are the same.

91. The therapeutic composition according to any one of claims 82-88, wherein at least two of the peptides harboured by the MHC molecules are different.

92. The therapeutic composition according to any one of claims 82-88, wherein the peptides harboured by the MHC molecules are the same.

93. The therapeutic composition according to any one of claims 82-92, wherein the MHC molecules are attached to the carrier molecule directly.

94. The therapeutic composition according to any one of claims 82-92, wherein the MHC molecules are attached to the carrier molecule via one or more binding entities.

95. The therapeutic composition according to claim 94, wherein each binding entity has attached thereto from 1 to 10 MHC molecules.

96. The therapeutic composition according to claim 94, wherein each binding entity has attached thereto from 1 to 8 MHC molecules.

97. The therapeutic composition according to claim 94, wherein each binding entity has attached thereto from 1 to 6 MHC molecules.

98. The therapeutic composition according to claim 94, wherein each binding entity has attached thereto from 1 to 4 MHC molecules.

99. The therapeutic composition according to claim 94, wherein each binding entity has attached thereto from 1 to 3 MHC molecules.

100. The therapeutic composition according to claim 94, wherein each binding entity has attached thereto 1 or 2 MHC molecules.

101. The therapeutic composition according to any one of claims 82-100, wherein the total number of MHC molecules of the construct is from 1 to 100.

102. The therapeutic composition according to any one of claims 82-100, wherein the total number of MHC molecules of the construct is from 1 to 50.

103. The therapeutic composition according to any one of claims 82-100, wherein the total number of MHC molecules of the construct is from 1 to 25.

104. The therapeutic composition according to claim 94, wherein the binding entity is selected from streptavidin (SA) and avidin and derivatives thereof, biotin, immunoglobulins, antibodies (monoclonal, polyclonal, and recombinant), antibody fragments and derivatives thereof, leucine zipper domain of AP-1 (jun and fos), hexa-his (metal chelate moiety), hexa-hat GST (glutathione S-tranferase) glutathione affinity, Calmodulin-binding peptide (CBP), Strep-tag, Cellulose Binding Domain, Maltose Binding Protein, S-Peptide Tag, Chitin Binding Tag, Immuno-reactive Epitopes, Epitope Tags, E2Tag, HA Epitope Tag, Myc Epitope, FLAG Epitope, AU1 and AU5 Epitopes, Glu-Glu Epitope, KT3 Epitope, IRS Epitope, Btag Epitope, Protein Kinase-C Epitope, VSV Epitope, lectins that mediate binding to a diversity of compounds, including carbohydrates, lipids and proteins, e.g. Con A

(*Canavalia ensiformis*) or WGA (wheat germ agglutinin) and tetranectin or Protein A or G (antibody affinity).

105. The therapeutic composition according to any one of claims 82-104 further comprising one or more biologically active molecules.

106. The therapeutic composition according to claim 105, wherein the biologically active molecules is selected from proteins, co-stimulatory molecules, cell modulating molecules, receptors, accessory molecules, adhesion molecules, natural ligands, and toxic molecules, and antibodies and recombinant binding molecules thereto, and combinations thereof.

107. The therapeutic composition according to claim 105 or 106, wherein the biologically active molecule is attached to the carrier molecule either directly or via one or more of the binding entities.

108. The therapeutic composition according to any one of claims 105-107, wherein the biologically active molecule is selected from

proteins such as MHC Class I-like proteins like MIC A, MIC B, CD1d, HLA E, HLA F, HLA G, HLA H, ULBP-1, ULBP-2, and ULBP-3,

co-stimulatory molecules such as CD2, CD3, CD4, CD5, CD8, CD9, CD27, CD28, CD30, CD69, CD134 (OX40), CD137 (4-1BB), CD147, CDw150 (SLAM), CD152 (CTLA-4), CD153 (CD30L), CD40L (CD154), NKG2D, ICOS, HVEM, HLA Class II, PD-1, Fas (CD95), FasL expressed on T and/or NK cells, CD40, CD48, CD58, CD70, CD72, B7.1 (CD80), B7.2 (CD86), B7RP-1, B7-H3, PD-L1, PD-L2, CD134L, CD137L, ICOSL, LIGHT expressed on APC and/or tumour cells,

cell modulating molecules such as CD16, NKp30, NKp44, NKp46, NKp80, 2B4, KIR, LIR, CD94/NKG2A, CD94/NKG2C expressed on NK cells, IFN-alpha, IFN-beta, IFN-gamma, IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, CSFs (colony-stimulating factors), vitamin D3, IL-2 toxins, cyclosporin, FK-506, rapamycin, TGF-beta, clotrimazole, nitrendipine, and charybdotoxin,

accessory molecules such as LFA-1, CD11a/18, CD54 (ICAM-1), CD106 (VCAM), and CD49a,b,c,d,e,f/CD29 (VLA-4),

adhesion molecules such as ICAM-1, ICAM-2, GlyCAM-1, CD34, anti-LFA-1, anti-CD44, anti-beta7, chemokines, CXCR4, CCR5, anti-selectin L, anti-selectin E, and anti-selectin P,

toxic molecules such as cyclophosphamide, methrotrexate, Azathioprine, mizoribine, 15-deoxuspergualin, neomycin, staurosporine, genestein, herbimycin A, Pseudomonas exotoxin A, saporin, Rituxan, Ricin, gemtuzumab ozogamicin, Shiga toxin, heavy metals like inorganic and organic mercurials, and FN18-CRM9, radioisotopes such as incorporated isotopes of iodide, cobalt, selenium, tritium, and phosphor, and haptens such as DNP, and digoxiginin,

and antibodies thereto, or antibody derivatives or fragments thereof, and combinations thereof.

109. The therapeutic composition according to any one of claims 82-108, wherein the carrier molecule is selected from

polysaccharides including dextrans, carboxy methyl dextran, dextran polyaldehyde, carboxymethyl dextran lactone, and cyclodextrins,

pullulans, schizophyllan, scleroglucan, xanthan, gellan, O-ethylamino guaran, chitins and chitosans indlucing 6-O-carboxymethyl chitin and N-carboxymethyl chitosan,

derivatised cellolosics including carboxymethyl cellulose, carboxymethyl hydroxyethyl cellulose, hydroxy-ethyl cellulose, 6-amino-6-deoxy cellulose and O-ethyl-amine cellulose,

hydroxylated starch, hydroxypropyl starch, hydroxyethyl starch, carrageenans, alginates, and agarose,

synthetic polysaccharides including ficoll and carboxy-methylated ficoll,

vinyl polymers including poly(acrylic acid), poly(acryl amides), poly(acrylic esters), poly(2-hydroxy ethyl meth-acrylate), poly(methyl methacrylate), poly(maleic acid), poly(maleic anhydride), , poly(acrylamide), poly(ethyl-co-vinyl acetate), poly(methacrylic acid), poly(vinyl-alcohol), poly(vinyl alcohol-co-vinyl chloroacetate), aminated poly(vinyl alcohol), and co block polymers thereof,

poly ethylene glycol (PEG) or polypropylene glycol or poly(ethylene oxide-co-propylene oxides) containing polymer backbones including linear, comb-shaped or StarBurst™ dendrimers,

poly amino acids including polylysines, polyglutamic acid, polyurethanes, poly(ethylene imines), pluriol.

proteins including albumins, immunoglobulins, and virus-like proteins (VLP), and

polynucleotides, DNA, PNA, LNA, oligonucleotides and oligonucleotide dendrimer constructs.

110. The therapeutic composition according to any one of claims 82-109, wherein the carrier molecule is a soluble carrier molecule.

111. The therapeutic composition according to any one of claims 82-110 further comprising one or more adjuvants and/or excipients.

112. The therapeutic composition according to claim 111, wherein the adjuvant is selected from saponins such as Quil A and Qs-21, oil in water emulsions such as MF59, MPL, PLG, PLGA, aluminium salts, calcium phosphate, water in oil emulsions such as IFA (Freund's incomplete adjuvant) and CFA (Freund's complete adjuvant), interleukins such as IL-1β, IL-2, IL-7, IL-12, and INFγ, Adju-Phos®, glucan, antigen formulation, biodegradable microparticles, Cholera Holotoxin, liposomes, DDE, DHEA, DMPC, DMPG, DOC/Alum Complex, ISCOMs®, muramyl dipeptide, monophosphoryl lipid A, muramyl tripeptide, and phospatidylethanolamine In a preferred embodiment, the adjuvant is selected from saponins such as Quil A and Qs-21, MF59, MPL, PLG, PLGA, calcium phosphate, and aluminium salts.

113. The therapeutic composition according to claim 113, wherein the excipient is selected from diluents, buffers, suspending agents, wetting agents, solubilising agents, pH-adjusting agents, dispersing agents, preserving agents, and/or colorants.

114. The therapeutic composition according to any one of claims 82-113 for the treatment, prevention, stabilisation, or alleviation of disease involving MHC recognising cells.

115. The therapeutic composition according to claim 114, wherein the MHC recognising cells are involved in a disease of inflammatory, auto-immune, allergic, viral, cancerous, infectious, allo- or xenogene (graft versus host and host versus graft) origin.

116. The therapeutic composition according to claim 115, wherein the disease is a chronic inflammatory bowel disease such as Crohn's disease or ulcerative colitis, sclerosis, type I diabetes, rheumatoid arthritis, psoriasis, atopic dermatitis, asthma, malignant melanoma, renal carcinoma, breast cancer, lung cancer, cancer of the uterus, prostatic cancer, brain cancer, head and neck cancer, leukaemia, cutaneous lymphoma, hepatic carcinoma, colorectal cancer, bladder cancer, rejection-related disease, Graft-versus-host-related disease, or a viral disease associated with hepatitis, AIDS, measles, pox, chicken pox, rubella or herpes.

117. The therapeutic composition according to any one of claims 82-116 formulated for parenteral administration, including intravenous, intramuscular, intraarticular, subcutaneous, intradermal, epicutantous/transdermal, and intraperitoneal administration, for infusion, for oral administration, for nasal administration, for rectal administration, or for topic administration.

118. A therapeutic composition comprising as active ingredient an effective amount of MHC recognising cells, the MHC recognising cells being obtained by

bringing a sample from a subject comprising MHC recognising cells into contact with a MHC molecule construct according to any one of claims 1-42, whereby the MHC recognising cells become bound to the MHC molecule construct,

isolating the bound MHC molecule construct and the MHC recognising cells, and

expanding such MHC recognising cells to a clinically relevant number.

119. The therapeutic composition according to claim 118, wherein the isolated MHC recognising cells are liberated from the MHC molecule construct prior to expansion.

120. The therapeutic composition according to claims 118 or 119, wherein the MHC molecule construct is immobilised onto a solid or semi-solid support.

121. The therapeutic composition according to claim 120, wherein the MHC molecule construct is immobilised onto the solid or semi-solid support prior to contact with the sample.

122. The therapeutic composition according to claim 120, wherein the MHC molecule construct is immobilised onto the solid or semi-solid support following contact with the sample.

123. The therapeutic composition according to any one of claims 118-122, wherein the expansion is carried out in the presence of one or more MHC molecule constructs, optionally one or more biologically active molecules and optionally feeder cells such as dendritic cells or feeder cells.

124. The therapeutic composition according to any one of claims 120-123, wherein the MHC molecule construct is immobilised onto the solid or semi-solid support directly.

125. The therapeutic composition according to any one of claims 120-124, wherein the MHC molecule construct is immobilised to the solid or semi-solid support via a linker, a spacer, or an antibody, an antibody derivative or a fragment thereof.

126. The therapeutic composition according to any one of claims 120-125, wherein the solid or semi-solid support is selected from particles, beads, biodegradable particles, sheets, gels, filters, membranes, fibres, capillaries, needles, microtitre strips, tubes, plates or wells, combs, pipette tips, micro arrays, chips, and microtiter plates having one or more wells.

127. The therapeutic composition according to any one of claims 120-126, wherein the solid support is selected from particles and beads.

128. The therapeutic composition according to claim 127, wherein the particles and beads are polymeric, magnetic or superparamagnetic.

129. The therapeutic composition according to any one of claims 118-128, wherein the isolation is performed by applying a magnetic field or by flow cytometry.

130. The therapeutic composition according to any one of claims 118-128, wherein the MHC molecule construct comprises

a carrier molecule having attached thereto one or more MHC molecules, said MHC molecules being attached to the carrier molecule either directly or via one or more binding entities.

131. The therapeutic composition according to any one of claims 118-130, wherein the MHC molecule is a vertebrate MHC molecule such as a human, a murine, a rat, a porcine, a bovine or an avian molecule.

132. The therapeutic composition according to any one of claims 118-131, wherein the MHC molecule is a human MHC molecule.

133. The therapeutic composition according to any one of claims 118-132, wherein the MHC molecule is

a MHC Class I molecule selected from the group consisting of a heavy chain, a heavy chain combined with a β_2m, a heavy chain combined with a peptide, and a heavy chain/β_2m dimer with a peptide;

or a MHC Class II molecule selected from the group consisting of an α/β dimer, an α/β dimer with a peptide, α/β dimer combined through an affinity tag and a α/β dimer combined through an affinity tag with a peptide;

or a MHC Class I like molecule or a MHC Class II molecule.

134. The therapeutic composition according to any one of claims 118-133, wherein the MHC molecule is a peptide free MHC molecule.

135. The therapeutic composition according to any one of claims 118-134, wherein at least two of the MHC molecules are different.

136. The therapeutic composition according to any one of claims 118-135, wherein the MHC molecules are the same.

137. The therapeutic composition according to any one of claims 118-136, wherein at least two of the peptides harboured by the MHC molecules are different.

138. The therapeutic composition according to any one of claims 118-137, wherein the peptides harboured by the MHC molecules are the same.

139. The therapeutic composition according to any one of claims 118-138, wherein the MHC molecules are attached to the carrier molecule directly.

140. The therapeutic composition according to any one of claims 118-138, wherein the MHC molecules are attached to the carrier molecule via one or more binding entities.

141. The therapeutic composition according to claim 140, wherein each binding entity has attached thereto from 1 to 10 MHC molecules.

142. The therapeutic composition according to claim 140, wherein each binding entity has attached thereto from 1 to 8 MHC molecules.

143. The therapeutic composition according to claim 140, wherein each binding entity has attached thereto from 1 to 6 MHC molecules.

144. The therapeutic composition according to claim 140, wherein each binding entity has attached thereto from 1 to 4 MHC molecules.

145. The therapeutic composition according to claim 140, wherein each binding entity has attached thereto from 1 to 3 MHC molecules.

146. The therapeutic composition according to claim 140, wherein each binding entity has attached thereto 1 or 2 MHC molecules.

147. The therapeutic composition according to any one of claims 118-146, wherein the total number of MHC molecules of the construct is from 1 to 100.

148. The therapeutic composition according to any one of claims 118-146, wherein the total number of MHC molecules of the construct is from 1 to 50.

149. The therapeutic composition according to any one of claims 118-146, wherein the total number of MHC molecules of the construct is from 1 to 25.

150. The therapeutic composition according to claim 140, wherein the binding entity is selected from streptavidin streptavidin (SA) and avidin and derivatives thereof, biotin, immunoglobulins, antibodies (monoclonal, polyclonal, and recombinant), antibody fragments and derivatives thereof, leucine zipper domain of AP-1 (jun and fos), hexa-his (metal chelate moiety), hexa-hat GST (glutathione S-tranferase) glutathione affinity, Calmodulin-binding peptide (CBP), Strep-tag, Cellulose Binding Domain, Maltose Binding Protein, S-Peptide Tag, Chitin Binding Tag, Immuno-reactive Epitopes, Epitope Tags, E2Tag, HA Epitope Tag, Myc Epitope, FLAG Epitope,

AU1 and AU5 Epitopes, Glu-Glu Epitope, KT3 Epitope, IRS Epitope, Btag Epitope, Protein Kinase-C Epitope, VSV Epitope, lectins that mediate binding to a diversity of compounds, including carbohydrates, lipids and proteins, e.g. Con A (*Canavalia ensiformis*) or WGA (wheat germ agglutinin) and tetranectin or Protein A or G (antibody affinity).

151. The therapeutic composition according to any one of claims 118-150 further comprising one or more biologically active molecules.

152. The therapeutic composition according to claim 151, wherein the biologically active molecules is selected from proteins, co-stimulatory molecules, cell modulating molecules, receptors, accessory molecules, adhesion molecules, natural ligands, and toxic molecules, and antibodies and recombinant binding molecules thereto, and combinations thereof.

153. The therapeutic composition according to claim 150 or 151, wherein the biologically active molecule is attached to the carrier molecule either directly or via one or more of the binding entities.

154. The therapeutic composition according to any one of claims 151-153, wherein the biologically active molecule is selected from

proteins such as MHC Class I-like proteins like MIC A, MIC B, CD1d, HLA E, HLA F, HLA G, HLA H, ULBP-1, ULBP-2, and ULBP-3,

co-stimulatory molecules such as CD2, CD3, CD4, CD5, CD8, CD9, CD27, CD28, CD30, CD69, CD134 (OX40), CD137 (4-1BB), CD147, CDw150 (SLAM), CD152 (CTLA-4), CD153 (CD30L),

CD40L (CD154), NKG2D, ICOS, HVEM, HLA Class II, PD-1, Fas (CD95), FasL expressed on T and/or NK cells, CD40, CD48, CD58, CD70, CD72, B7.1 (CD80), B7.2 (CD86), B7RP-1, B7-H3, PD-L1, PD-L2, CD134L, CD137L, ICOSL, LIGHT expressed on APC and/or tumour cells,

cell modulating molecules such as CD16, NKp30, NKp44, NKp46, NKp80, 2B4, KIR, LIR, CD94/NKG2A, CD94/NKG2C expressed on NK cells, IFN-alpha, IFN-beta, IFN-gamma, IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, CSFs (colony-stimulating factors), vitamin D3, IL-2 toxins, cyclosporin, FK-506, rapamycin, TGF-beta, clotrimazole, nitrendipine, and charybdotoxin,

accessory molecules such as LFA-1, CD11a/18, CD54 (ICAM-1), CD106 (VCAM), and CD49a,b,c,d,e,f/CD29 (VLA-4),

adhesion molecules such as ICAM-1, ICAM-2, GlyCAM-1, CD34, anti-LFA-1, anti-CD44, anti-beta7, chemokines, CXCR4, CCR5, anti-selectin L, anti-selectin E, and anti-selectin P,

toxic molecules such as cyclophosphamide, methrotrexate, Azathioprine, mizoribine, 15-deoxuspergualin, neomycin, staurosporine, genestein, herbimycin A, Pseudomonas exotoxin A, saporin, Rituxan, Ricin, gemtuzumab ozogamicin, Shiga toxin, heavy metals like inorganic and organic mercurials, and FN18-CRM9, radioisotopes such as incorporated isotopes of iodide, cobalt, selenium, tritium, and phosphor, and haptens such as DNP, and digoxiginin,

and antibodies thereto, or antibody derivatives or fragments thereof, and combinations thereof.

155. The therapeutic composition according to any one of claims 118-154, wherein the carrier molecule is selected from

polysaccharides including dextrans, carboxy methyl dextran, dextran polyaldehyde, carboxymethyl dextran lactone, and cyclodextrins,

pullulans, schizophyllan, scleroglucan, xanthan, gellan, O-ethylamino guaran, chitins and chitosans indlucing 6-O-carboxymethyl chitin and N-carboxymethyl chitosan,

derivatised cellolosics including carboxymethyl cellulose, carboxymethyl hydroxyethyl cellulose, hydroxy-ethyl cellulose, 6-amino-6-deoxy cellulose and O-ethyl-amine cellulose,

hydroxylated starch, hydroxypropyl starch, hydroxyethyl starch, carrageenans, alginates, and agarose,

synthetic polysaccharides including ficoll and carboxy-methylated ficoll,

vinyl polymers including poly(acrylic acid), poly(acryl amides), poly(acrylic esters), poly(2-hydroxy ethyl meth-acrylate), poly(methyl methacrylate), poly(maleic acid), poly(maleic anhydride), , poly(acrylamide), poly(ethyl-co-vinyl acetate), poly(methacrylic acid), poly(vinyl-alcohol), poly(vinyl alcohol-co-vinyl chloroacetate), aminated poly(vinyl alcohol), and co block polymers thereof,

poly ethylene glycol (PEG) or polypropylene glycol or poly(ethylene oxide-co-propylene oxides) containing polymer backbones including linear, comb-shaped or StarBurst™ dendrimers,

poly amino acids including polylysines, polyglutamic acid, polyurethanes, poly(ethylene imines), pluriol.

proteins including albumins, immunoglobulins, and virus-like proteins (VLP), and

polynucleotides, DNA, PNA, LNA, oligonucleotides and oligonucleotide dendrimer constructs.

156. The therapeutic composition according to any one of claims 118-155 further comprising one or more labelling compounds.

157. The therapeutic composition according to claim 156, wherein one or more labelling compounds are attached to the carrier molecule.

158. The therapeutic composition according to claim 156, wherein one or more labelling compounds are attached to one or more of the binding entities.

159. The therapeutic composition according to claim 156, wherein one or more labelling compounds are attached to one or more of the MHC molecules.

160. The therapeutic composition according to claim 156, wherein one or more labelling compounds are attached to the carrier molecule and/or one or more of the binding entities and/or one or more of the MHC molecules.

161. The therapeutic composition according to any one of claims 156-160, wherein the labelling compound is directly or indirectly detectable.

162. The therapeutic composition according to any one of claims 156-161, wherein the labelling compound is a fluorescent label, an enzyme label, a radioisotope, a chemiluminescent label, a bioluminescent label, a polymer, a metal particle, a hapten, an antibody, or a dye.

163. The therapeutic composition according to any one of claims 156-162, wherein the labelling compound

is selected from fluorescent labels such as 5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate (FITC), rhodamine, tetramethylrhodamine, and dyes such as Cy2, Cy3, and Cy5, optionally substituted coumarin including AMCA, PerCP, phycobiliproteins including R-phycoerythrin (RPE) and allophycoerythrin (APC), Texas Red, Princeston Red, Green fluorescent protein (GFP) and analogues thereof, and conjugates of R-phycoerythrin or allophycoerythrin and e.g. Cy5 or Texas Red, and inorganic fluorescent labels based on semiconductor nanocrystals (like quantum dot and Qdot™ nanocrystals), and time-resolved fluorescent labels based on lanthanides like Eu3+ and Sm3+,

from haptens such as DNP, biotin, and digoxiginin, or

is selected from haptens such as DNP, fluorescein isothiocyanate (FITC), biotin, and digoxiginin, or

is selected from enzymatic labels such as horse radish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase (GAL), glucose-6-phosphate dehydrogenase, beta-N-acetylglucosaminidase, ß-glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO), or

is selected from luminiscence labels such as luminol, isoluminol, acridinium esters, 1,2-dioxetanes and pyridopyridazines, or

is selected from radioactivity labels such as incorporated isotopes of iodide, cobalt, selenium, tritium, and phosphor.

164. The therapeutic composition according to any one of claims 118-163, wherein the carrier molecule is a soluble carrier molecule.

165. The therapeutic composition according to any one of claims 118-164 further comprising one or more excipients.

166. The therapeutic composition according to claims 165, wherein the excipient is selected from diluents, buffers, suspending agents, wetting agents, solubilising agents, pH-adjusting agents, dispersing agents, preserving agents, and/or colorants.

167. The therapeutic composition according to any one of claims 118-166 for the treatment, prevention, stabilisation, or alleviation of a disease involving MHC recognising cells.

168. The therapeutic composition according to claim 167, wherein MHC recognising cells are involved in a disease of inflammatory, auto-immune, allergic, viral, cancerous, infectious, allo- or xenogene (graft versus host and host versus graft) origin.

169. The therapeutic composition according to claim 167 or 168, wherein the disease is a chronic inflammatory bowel disease such as Crohn's disease or ulcerative

colitis, sclerosis, type I diabetes, rheumatoid arthritis, psoriasis, atopic dermatitis, asthma, malignant melanoma, renal carcinoma, breast cancer, lung cancer, cancer of the uterus, prostatic cancer, brain cancer, head and neck cancer, leukaemia, cutaneous lymphoma, hepatic carcinoma, colorectal cancer, bladder cancer, rejection-related disease, Graft-versus-host-related disease, or a viral disease associated with hepatitis, AIDS, measles, pox, chicken pox, rubella or herpes.

170. The therapeutic composition according to any one of claims 118-169 formulated for parenteral administration, including intravenous, intramuscular, intraarticular, subcutaneous, intradermal, epicutantous/transdermal, and intraperitoneal administration, for infusion, for oral administration, for nasal administration, for rectal administration, or for topic administration.

171. The therapeutic composition according to any one of claims 82-170 for use in in vivo therapy.

172. A method of treating an animal, including a human being, comprising administering a therapeutic composition according to any one of claims 82-170 in an effective amount.

173. A method of up-regulating, down-regulating, modulate an immune response in an animal, including a human being, comprising administering a therapeutic composition according to any one of claims 82-170 in an effective amount.

174. A method of inducing anergy of a cell in an animal, including a human being, comprising administering a

therapeutic composition according to any one of claims 82-170 in an effective amount.

175. An adoptive cellular immunotherapeutic method comprising administrating to an animal, including a human being, a therapeutic composition according to any one of claims 82-170.

176. A method of obtaining MHC recognising cells comprising

bringing into contact a MHC molecule construct according to any one of claims 1-42 and a sample suspected of comprising MHC recognising cells under conditions whereby the MHC recognising cells bind to the MHC molecule construct, and
isolating the bound MHC molecule construct and MHC recognising cells.

177. The method according to claim 176, wherein the isolation is carried out by applying a magnetic field or by flow cytometry.

178. A method for producing a therapeutic composition according to any one of claims 82-170, comprising

providing a MHC molecule construct as defined in claims 1-42,
solubilising or dispersing the MHC molecule construct in a medium suitable for therapeutic substances, and optionally adding other adjuvants and/or excipients.

179. A method for producing a therapeutic composition according to any one of claims 118-170, comprising

obtaining MHC recognising cells using a MHC molecule construct according to any one of claims 1-42,
expanding such MHC recognising cells to a clinically relevant number,
formulating the obtained cells in a medium suitable for administration, and
optionally adding adjuvants and/or excipients.

180. Use of a MHC molecule construct according to any one of claims 1-42 for ex vivo expansion of MHC recognising cells.

181. Use according to claim 180, wherein the MHC molecule construct is in soluble form.

182. Use according to claim 180, wherein the MHC molecule construct is immobilised onto a solid or semi-solid support.

183. Use according to claim 182, wherein the solid or semi-solid support is selected from particles, beads, biodegradable particles, sheets, gels, filters, membranes (e. g. nylon membranes), fibres, capillaries, needles, microtitre strips, tubes, plates or wells, combs, pipette tips, micro arrays, chips, and slides.

184. Use according to claim 182 or 183, wherein the solid or semi-solid support is selected from beads and particles.

185. Use according to claim 184, wherein the solid or semi-solid support is selected from polymeric, magnetic or superparamagnetic particles and beads.

186. Use according to any one of claims 180-185, wherein the MHC molecule construct further comprises one or more biologically active molecules.

187. Use according to any one of claims 180-186, wherein wherein the biologically active molecule is selected from

proteins such as MHC Class I-like proteins like MIC A, MIC B, CD1d, HLA E, HLA F, HLA G, HLA H, ULBP-1, ULBP-2, and ULBP-3,

co-stimulatory molecules such as CD2, CD3, CD4, CD5, CD8, CD9, CD27, CD28, CD30, CD69, CD134 (OX40), CD137 (4-1BB), CD147, CDw150 (SLAM), CD152 (CTLA-4), CD153 (CD30L), CD40L (CD154), NKG2D, ICOS, HVEM, HLA Class II, PD-1, Fas (CD95), FasL expressed on T and/or NK cells, CD40, CD48, CD58, CD70, CD72, B7.1 (CD80), B7.2 (CD86), B7RP-1, B7-H3, PD-L1, PD-L2, CD134L, CD137L, ICOSL, LIGHT expressed on APC and/or tumour cells,

cell modulating molecules such as CD16, NKp30, NKp44, NKp46, NKp80, 2B4, KIR, LIR, CD94/NKG2A, CD94/NKG2C expressed on NK cells, IFN-alpha, IFN-beta, IFN-gamma, IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, IL-10, IL-11, IL-12, IL-15, CSFs (colony-stimulating factors), vitamin D3, IL-2 toxins, cyclosporin, FK-506, rapamycin, TGF-beta, clotrimazole, nitrendipine, and charybdotoxin,

accessory molecules such as LFA-1, CD11a/18, CD54 (ICAM-1), CD106 (VCAM), and CD49a,b,c,d,e,f/CD29 (VLA-4),

adhesion molecules such as ICAM-1, ICAM-2, GlyCAM-1, CD34, anti-LFA-1, anti-CD44, anti-beta7, chemokines, CXCR4, CCR5, anti-selectin L, anti-selectin E, and anti-selectin P,

toxic molecules such as cyclophosphamide, methrotrexate, Azathioprine, mizoribine, 15-deoxuspergualin, neomycin, staurosporine, genestein, herbimycin A, Pseudomonas exotoxin A, saporin, Rituxan, Ricin, gemtuzumab ozogamicin, Shiga toxin, heavy metals like inorganic and organic mercurials, and FN18-CRM9, radioisotopes such as incorporated isotopes of iodide, cobalt, selenium, tritium, and phosphor, and haptens such as DNP, and digoxiginin,

and antibodies thereto, or antibody derivatives or fragments thereof, and combinations thereof.

188. Use of a MHC molecule in a histological method.

189. Use of a MHC molecule in a cytological method.

190. Use of a MHC molecule according to claim 188 or 189 in a method for determining the presence of MHC recognising cells in a sample, in which method the MHC recognising cells of the sample are mounted on a support.

191. Use of a MHC molecule according to claim 188 or 189, in a method for monitoring the presence of MHC recognising cells in a sample, in which method the MHC recognising cells of the sample are mounted on a support.

192. Use of a MHC molecule according to claim 188 or 189 in a method for determining the status of a disease involving MHC recognising cells, in which method the MHC recognising cells of the sample are mounted on a support.

193. Use of a MHC molecule according to claim 188 or 189 in a method for establishing a prognosis of a disease involving MHC recognising cells, in which method the MHC recognising cells of the sample are mounted on a support.

194. Use of a MHC molecule according to any one of claims 188-193, wherein the support is a solid or semi-solid support.

195. Use of a MHC molecule according to any one of claims 188-194, wherein the support is selected from glass slides, membranes, filters, polymer slides, chamber slides, dishes, and petridishes.

196. Use according to any one of claims 188-195, wherein the sample is selected from histological material, cytological material, primary tumours, secondary organ metastasis, fine needle aspirates, spleen tissue, bone marrow specimens, cell smears, exfoliative cytological specimens, touch preparations, oral swabs, laryngeal swabs, vaginal swabs, bronchial lavage, gastric lavage, from the umbilical cord, and from body fluids such as blood (e.g. from a peripheral blood mononuclear cell (PBMC) population isolated from blood or from other blood-derived preparations such as leukopheresis products), from sputum samples, expectorates, and bronchial aspirates.

197. The use according to any one of claims 188-196, wherein the MHC molecule is

a MHC Class I molecule selected from the group consisting of a heavy chain, a heavy chain combined with a β_2m, a heavy chain combined with a peptide, and a heavy chain/β_2m dimer with a peptide;

or a MHC Class II molecule selected from the group consisting of an α/β dimer, an α/β dimer with a peptide, α/β dimer combined through an affinity tag and a α/β dimer combined through an affinity tag with a peptide;

or a MHC Class I like molecule or a MHC Class II like molecule.

198. The use according to any one of claims 188-197, wherein the MHC molecule is a vertebrate MHC molecule such as a human, a murine, a rat, a porcine, a bovine or an avian molecule.

199. The use according to any one of claims 188-198, wherein the MHC molecule is a human MHC molecule.

200. The use according to any one of claims 188-199, wherein the MHC molecule is a peptide free MHC molecule.

201. The use according to any one of claims 188-200, wherein the MHC molecule is attached to a binding entity.

202. Use according to claim 201, wherein the binding entity has attached thereto from 1 to 10 MHC molecules, such as from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 1 or 2 MHC molecules.

203. Use according to claim 201, wherein the binding entity is selected from streptavidin streptavidin (SA) and avidin and derivatives thereof, biotin, immunoglobulins, antibodies (monoclonal, polyclonal, and recombinant), antibody fragments and derivatives thereof, leucine zipper domain of AP-1 (jun and fos), hexa-his (metal chelate moiety), hexa-hat GST (glutathione S-tranferase) glutathione affinity, Calmodulin-binding peptide (CBP), Strep-tag, Cellulose Binding Domain, Maltose Binding Protein, S-Peptide Tag, Chitin Binding Tag, Immuno-reactive Epitopes, Epitope Tags, E2Tag, HA Epitope Tag, Myc Epitope, FLAG Epitope, AU1 and AU5

Epitopes, Glu-Glu Epitope, KT3 Epitope, IRS Epitope, Btag Epitope, Protein Kinase-C Epitope, VSV Epitope, lectins that mediate binding to a diversity of compounds, including carbohydrates, lipids and proteins, e.g. Con A (*Canavalia ensiformis*) or WGA (wheat germ agglutinin) and tetranectin or Protein A or G (antibody affinity).

204. Use according to any one of claims 188-203, wherein the MHC molecule further comprises a labelling compound.

205. Use according to claim 204, wherein the labelling compound can be detected directly or indirectly.

206. Use according to claim 204 or 205, wherein the labelling compound is a fluorescent label, an enzyme label, a radioisotope, a chemiluminescent label, a bioluminescent label, a polymer, a metal particle, a hapten, an antibody, or a dye.

207. Use according to any one of claims 204-206, wherein the labelling compound is selected from

5-(and 6)-carboxyfluorescein, 5- or 6-carboxyfluorescein, 6-(fluorescein)-5-(and 6)-carboxamido hexanoic acid, fluorescein isothiocyanate (FITC), rhodamine, tetramethylrhodamine, and dyes such as Cy2, Cy3, and Cy5, optionally substituted coumarin including AMCA, PerCP, phycobiliproteins including R-phycoerythrin (RPE) and allophycoerythrin (APC), Texas Red, Princeston Red, Green fluorescent protein (GFP) and analogues thereof, and conjugates of R-phycoerythrin or allophycoerythrin and e.g. Cy5 or Texas Red, and inorganic fluorescent labels based on semiconductor nanocrystals (like quantum dot and Qdot™ nanocrystals), and time-resolved fluorescent labels based on lanthanides like Eu3+ and Sm3+,

from haptens such as DNP, biotin, and digoxiginin,, or

is selected from enzymatic labels such as horse radish peroxidase (HRP), alkaline phosphatase (AP), beta-galactosidase (GAL), glucose-6-phosphate dehydrogenase, beta-N-acetylglucosaminidase, ß-glucuronidase, invertase, Xanthine Oxidase, firefly luciferase and glucose oxidase (GO), or

is selected from luminiscence labels such as luminol, isoluminol, acridinium esters, 1,2-dioxetanes and pyridopyridazines, or

is selected from radioactivity labels such as incorporated isotopes of iodide, cobalt, selenium, tritium, and phosphor.

208. The use according to any one of claims 204-207, wherein the labelling compound is attached to the MHC molecule and/or the binding entity.

209. A method for detecting the presence of MHC recognising cells in a sample comprising the steps of

(a) providing a sample suspected of comprising MHC recognising cells mounted on a support,
(b) contacting the sample with a MHC molecule as defined in claims 188-208, and
(c) determining any binding of the MHC molecule, which binding indicates the presence of MHC recognising cells.

210. A method for monitoring MHC recognising cells comprising the steps of

(a) providing a sample suspected comprising MHC recognising cells mounted on a support,

(b) contacting the sample with a MHC molecule as defined in claims 188-208, and
(c) determining any binding of the MHC molecule, thereby monitoring MHC recognising cells.

211. A method for the prognosis of a disease involving MHC recognising cells comprising the steps of

(a) providing a sample suspected comprising MHC recognising cells mounted on a support,
(b) contacting the sample with a MHC molecule as defined in claims 188-208, and
(c) determining any binding of the MHC molecule, thereby establishing a prognosis of a disease involving MHC recognising cells.

212. A method for determining the status of a disease involving MHC recognising cells comprising the steps of

(a) providing a sample suspected comprising MHC recognising cells mounted on a support,
(b) contacting the sample with a MHC molecule as defined in claims 188-208, and
(c) determining any binding of the MHC molecule, thereby determining the status of a disease involving MHC recognising cells.

213. A method for the diagnosis of a disease involving MHC recognising cells comprising the steps of

(a) providing a sample suspected comprising MHC recognising cells mounted on a support,
(b) contacting the sample with a MHC molecule as defined in claims 188-208, and
(c) determining any binding of the MHC molecule, thereby diagnosing a disease involving MHC recognising cells.

214. A method for the effectiveness of a medicament against a disease involving MHC recognising cells comprising the steps of

(a) providing a sample from a subject receiving treatment with a medicament mounted on a support,
(b) contacting the sample with a MHC molecule as defined in claims 188-208, and
(c) determining any binding of the MHC molecule, thereby determining the effectiveness of the medicament.

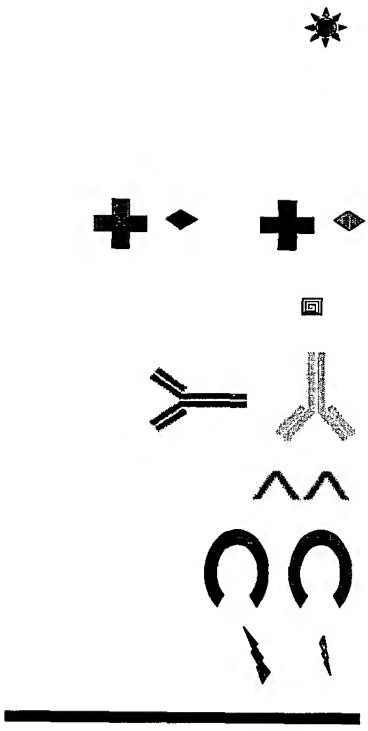
215. The method according to any one of claims 209-214, wherein the MHC recognising cells are involved in a disease of inflammatory, auto-immune, allergic, viral, cancerous, infectious, allo- or xenogene (graft-versus-host and host-versus-graft) origin.

216. The method according to claim 215, wherein the disease is a chronic inflammatory bowel disease such as Crohn's disease or ulcerative colitis, sclerosis, type I diabetes, rheumatoid arthritis, psoriasis, atopic dermatitis, asthma, malignant melanoma, renal carcinoma, breast cancer, lung cancer, cancer of the uterus, cervical cancer, prostatic cancer, brain cancer, head and neck cancer, leukaemia, cutaneous lymphoma, hepatic carcinoma, colorectal cancer, bladder cancer, rejection-related disease, Graft-versus-host-related disease, or a viral disease associated with hepatitis, AIDS, measles, pox, chicken pox, rubella or herpes.

217. The method according to any one of claims 209-215, wherein the MHC recognising cells are selected from subpopulations of CD3+ T-cells, gamma,delta T-cells, alpha,beta T-cells, CD4+ T-cells, T helper cels, CD8+ T-

cells, Suppressor T-cells, CD8+ cytotoxic T-cells, CTLs, NK cells, NKT cells, LAK cells, and MAK.

218. The method according to any one of claims 209-217, wherein the sample is selected from histological material, cytological material, primary tumours, secondary organ metastasis, fine needle aspirates, spleen tissue, bone marrow specimens, cell smears, exfoliative cytological specimens, touch preparations, oral swabs, laryngeal swabs, vaginal swabs, bronchial lavage, gastric lavage, from the umbilical cord, and from body fluids such as blood (e.g. from a peripheral blood mononuclear cell (PBMC) population isolated from blood or from other blood-derived preparations such as leukopheresis products), from sputum samples, expectorates, and bronchial aspirates.



Label
Direct label: Fluorescein, Alexa, Cy3, Cy5, Rhodamine, RPE, radioactive etc
Or
Indirect direct: HRP, AP, GO etc

Pair of binding entities (e.g. Streptavidine-Biotin or Jun-fos)

Hapten (e.g. DNP, Dig, biotin, FITC etc)

Antiboby, primary or secondary

Specific receptor or receptors

MHC class 1 or 2 molecules

Peptides

Polymer backbone

Figure 1

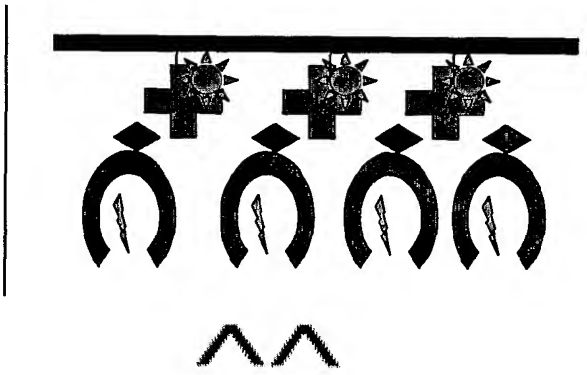


Figure 2

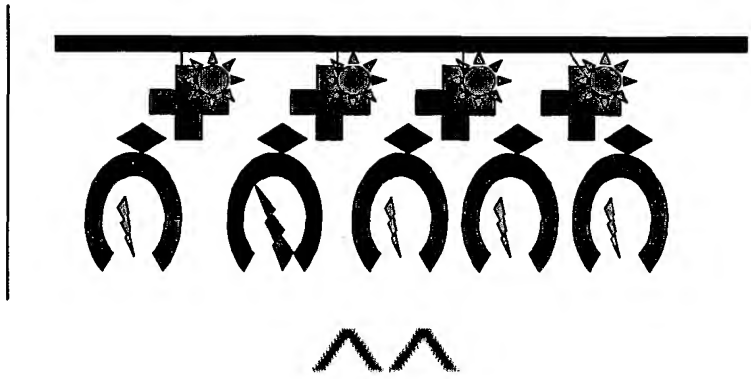


Figure 3

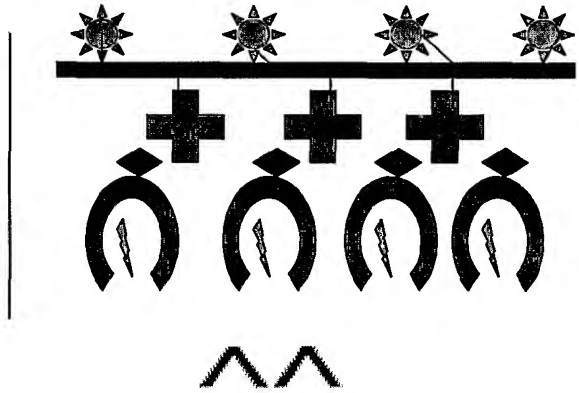


Figure 4

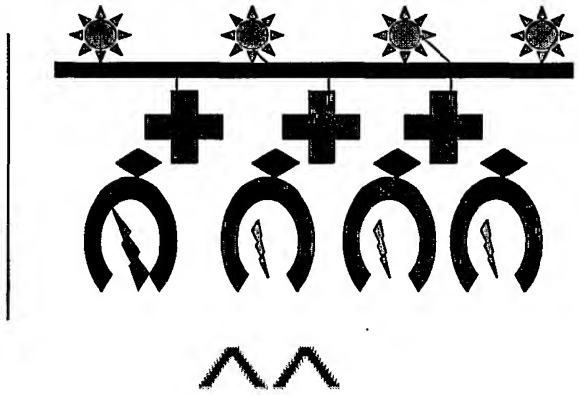


Figure 5

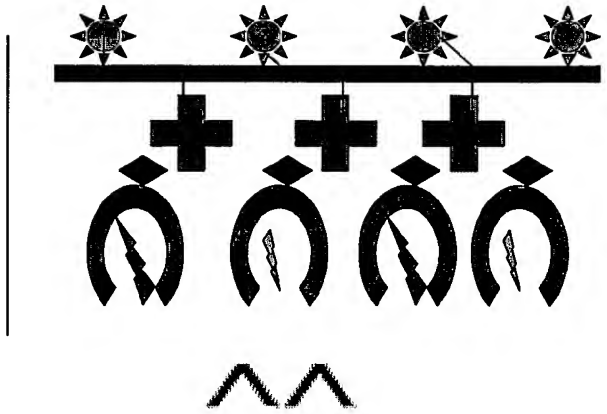


Figure 6

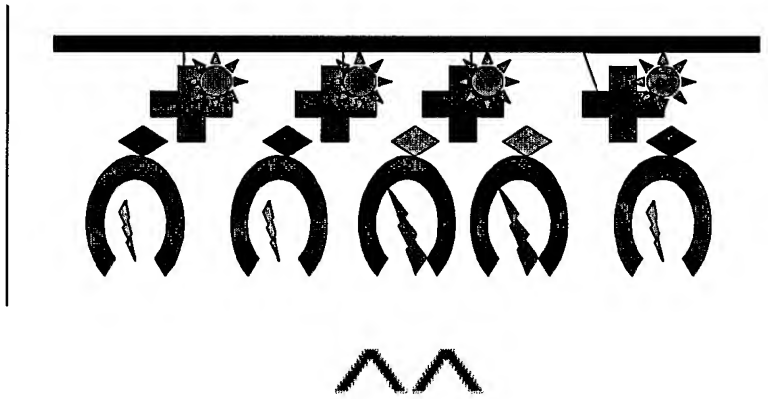


Figure 7

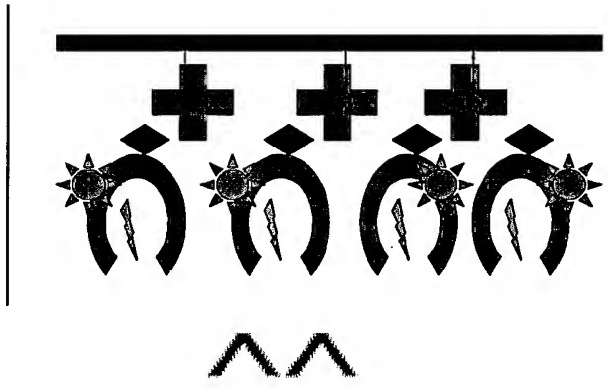


Figure 8

Figure 9

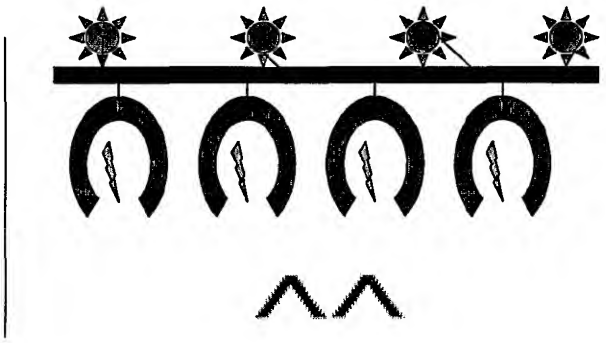


Figure 10

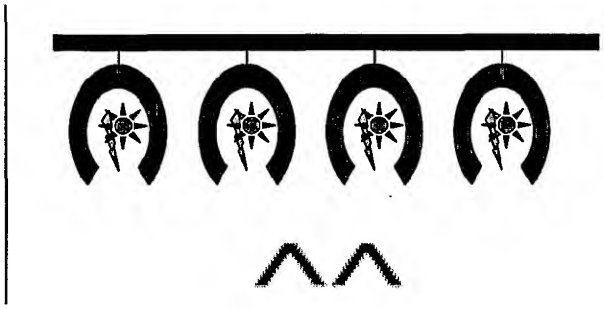


Figure 11

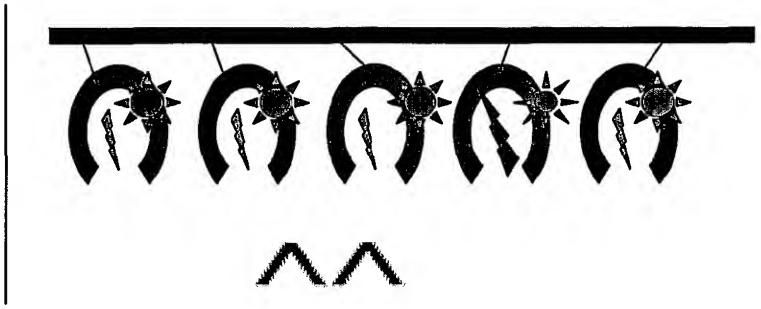


Figure 12

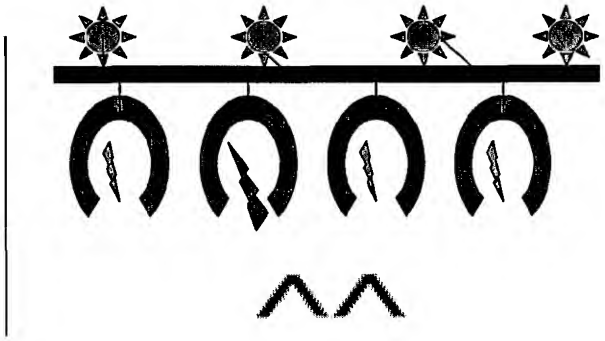


Figure 13

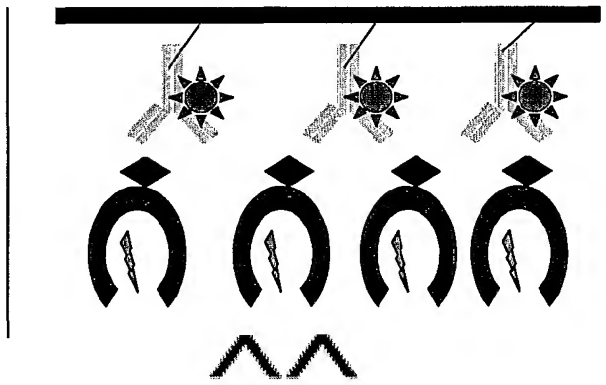


Figure 14

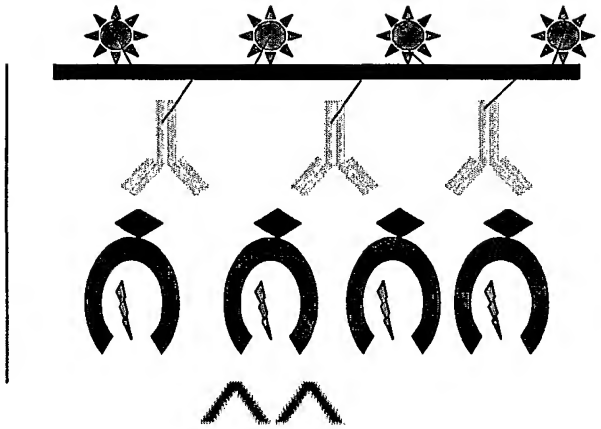


Figure 15

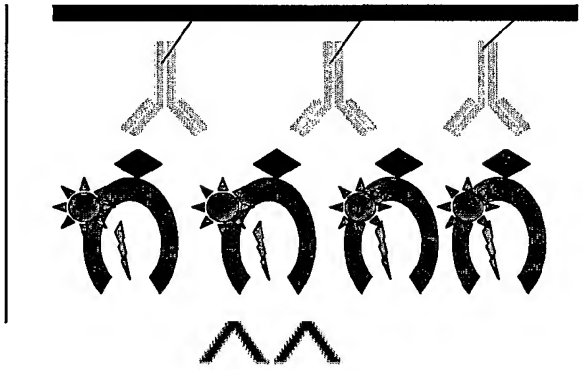


Figure 16

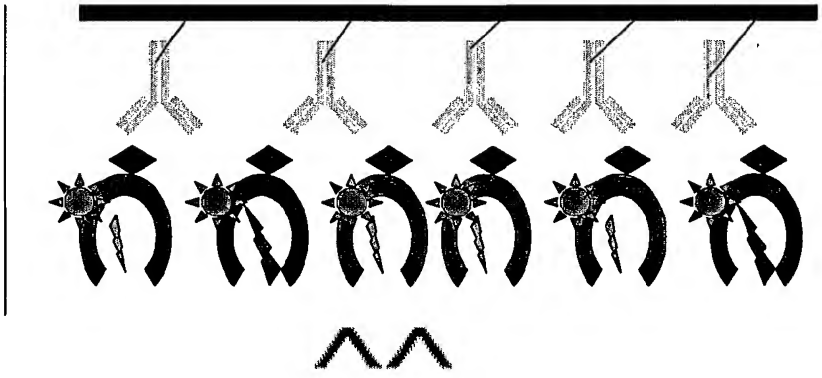


Figure 17

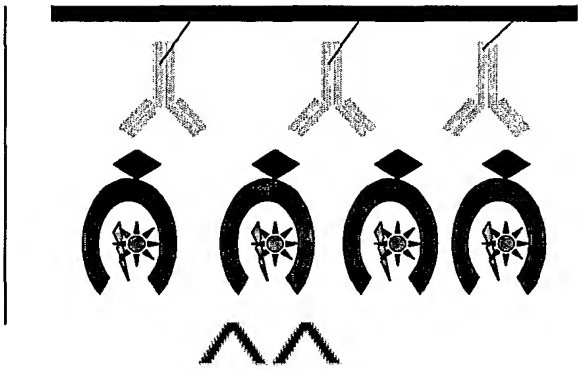


Figure 18

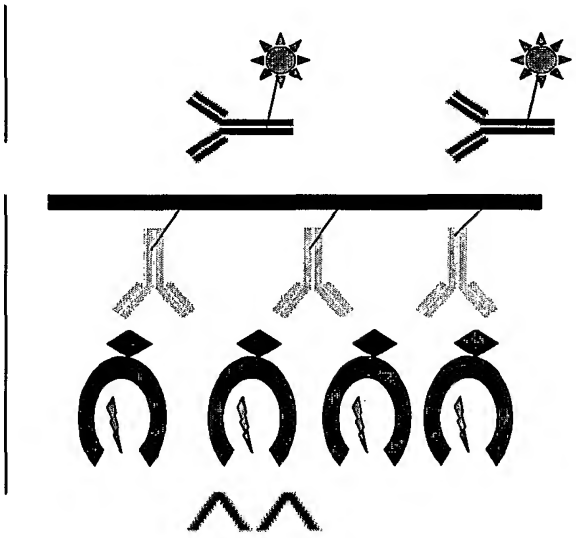


Figure 19

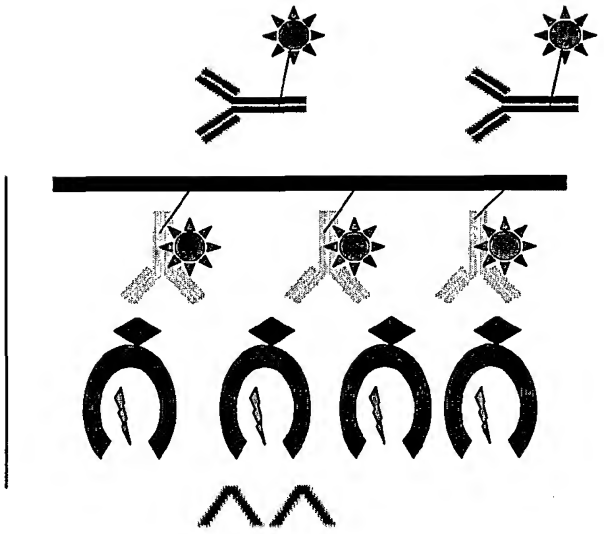


Figure 20

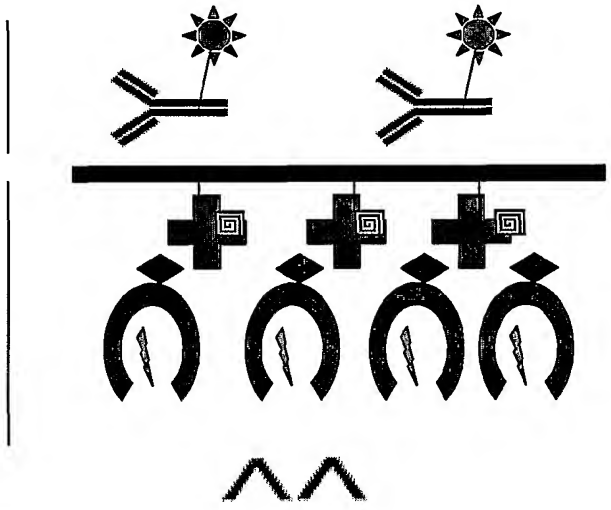


Figure 21

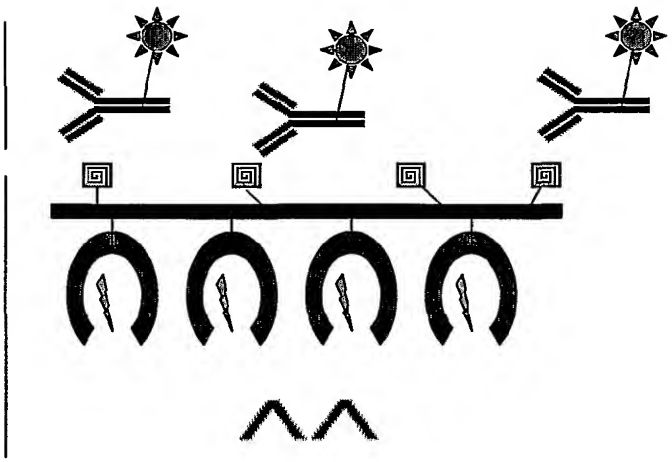


Figure 22

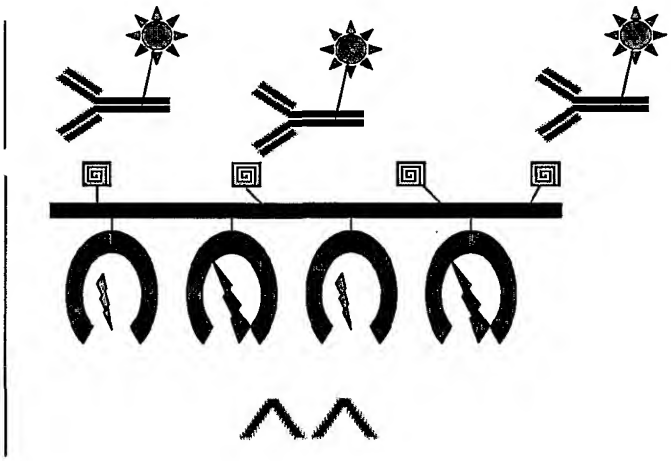


Figure 23

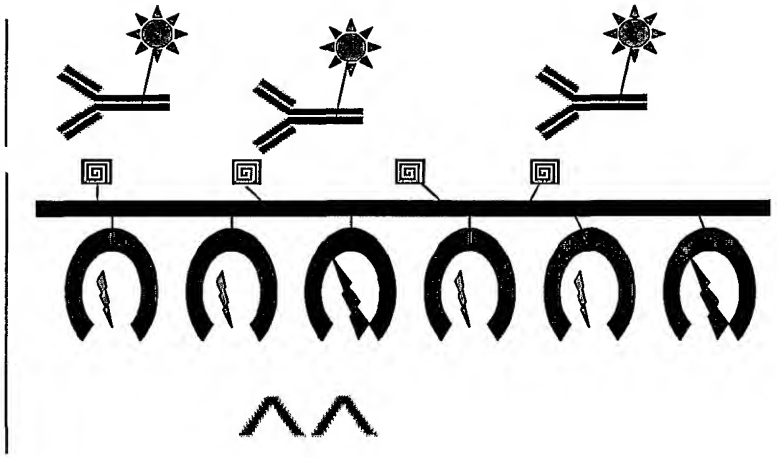
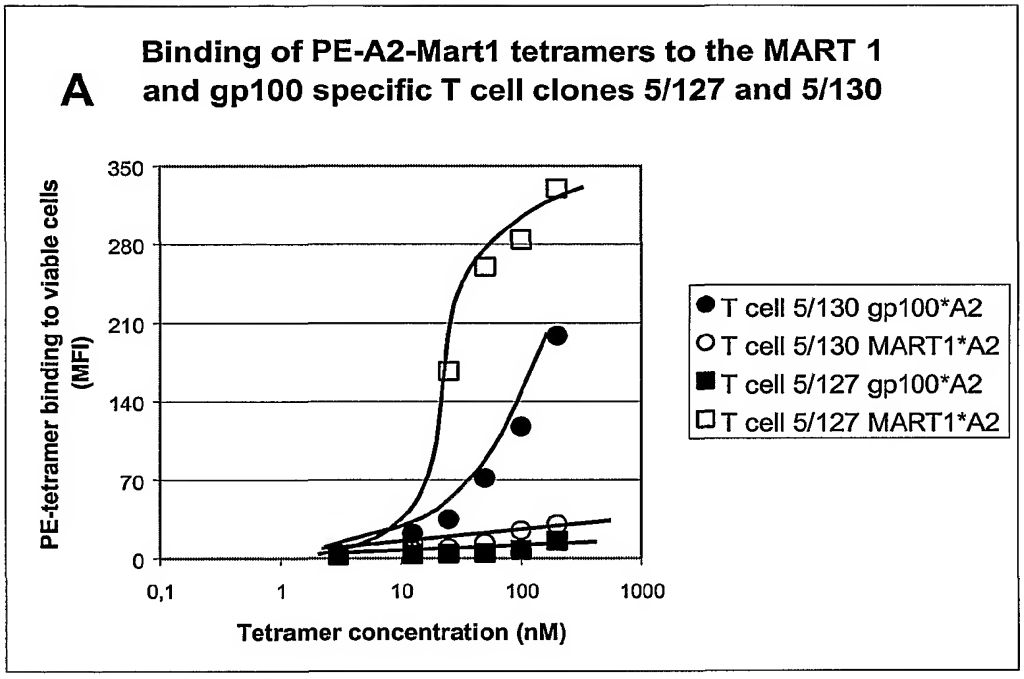


Figure 24


A
Binding of PE-A2-Mart1 tetramers to the MART 1 and gp100 specific T cell clones 5/127 and 5/130
PE-tetramer binding to viable cells (MFI)
350
280
210
140
70
0
0,1
1
10
100
1000
Tetramer concentration (nM)
T cell 5/130 gp100*A2
T cell 5/130 MART1*A2
T cell 5/127 gp100*A2
T cell 5/127 MART1*A2

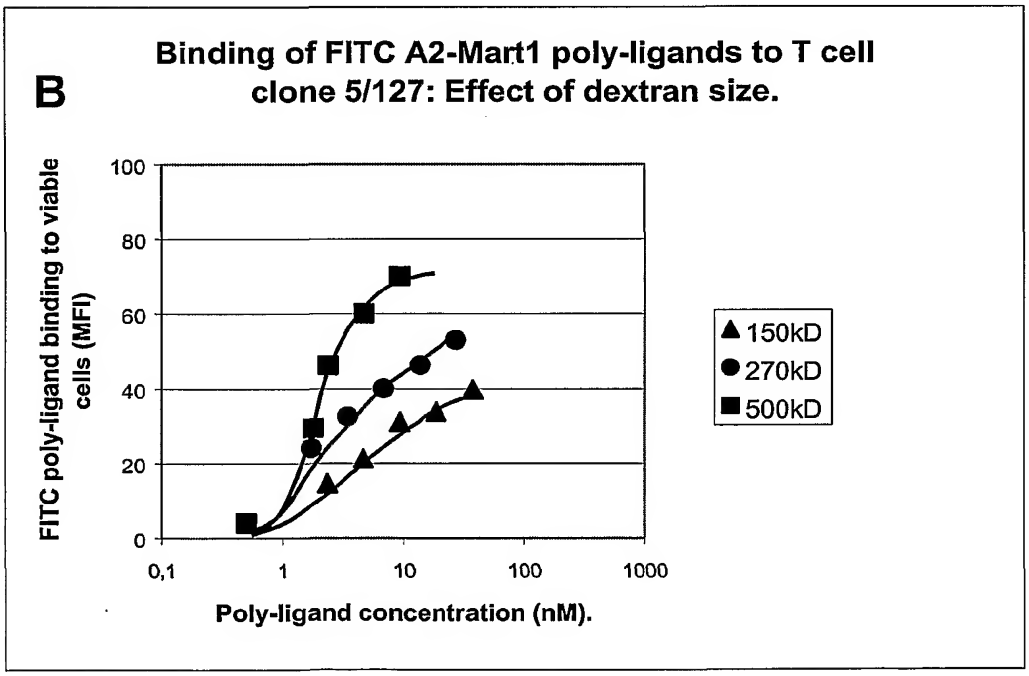

B
Binding of FITC A2-Mart1 poly-ligands to T cell clone 5/127: Effect of dextran size.
FITC poly-ligand binding to viable cells (MFI)
100
80
60
40
20
0
0,1
1
10
100
1000
Poly-ligand concentration (nM).
150kD
270kD
500kD

Figure 25

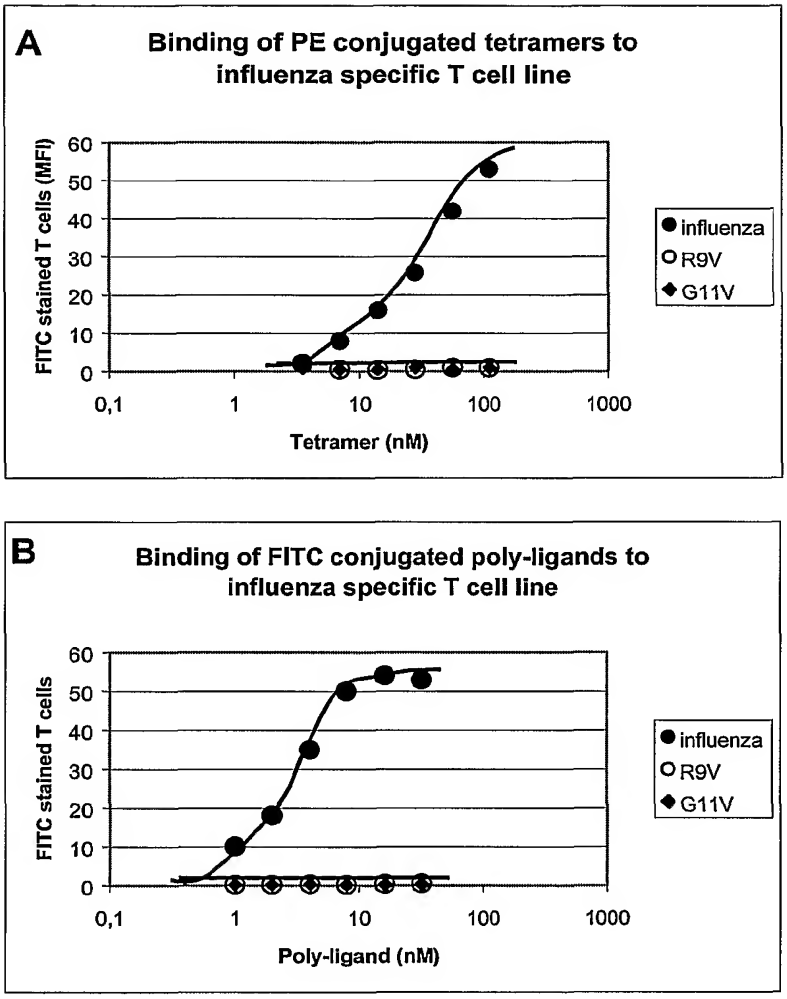

A
Binding of PE conjugated tetramers to influenza specific T cell line
FITC stained T cells (MFI)
60
50
40
30
20
10
0
0,1
1
10
100
1000
Tetramer (nM)
influenza
R9V
G11V
B
Binding of FITC conjugated poly-ligands to influenza specific T cell line
FITC stained T cells
60
50
40
30
20
10
0
0,1
1
10
100
1000
Poly-ligand (nM)
influenza
R9V
G11V

Figure 26

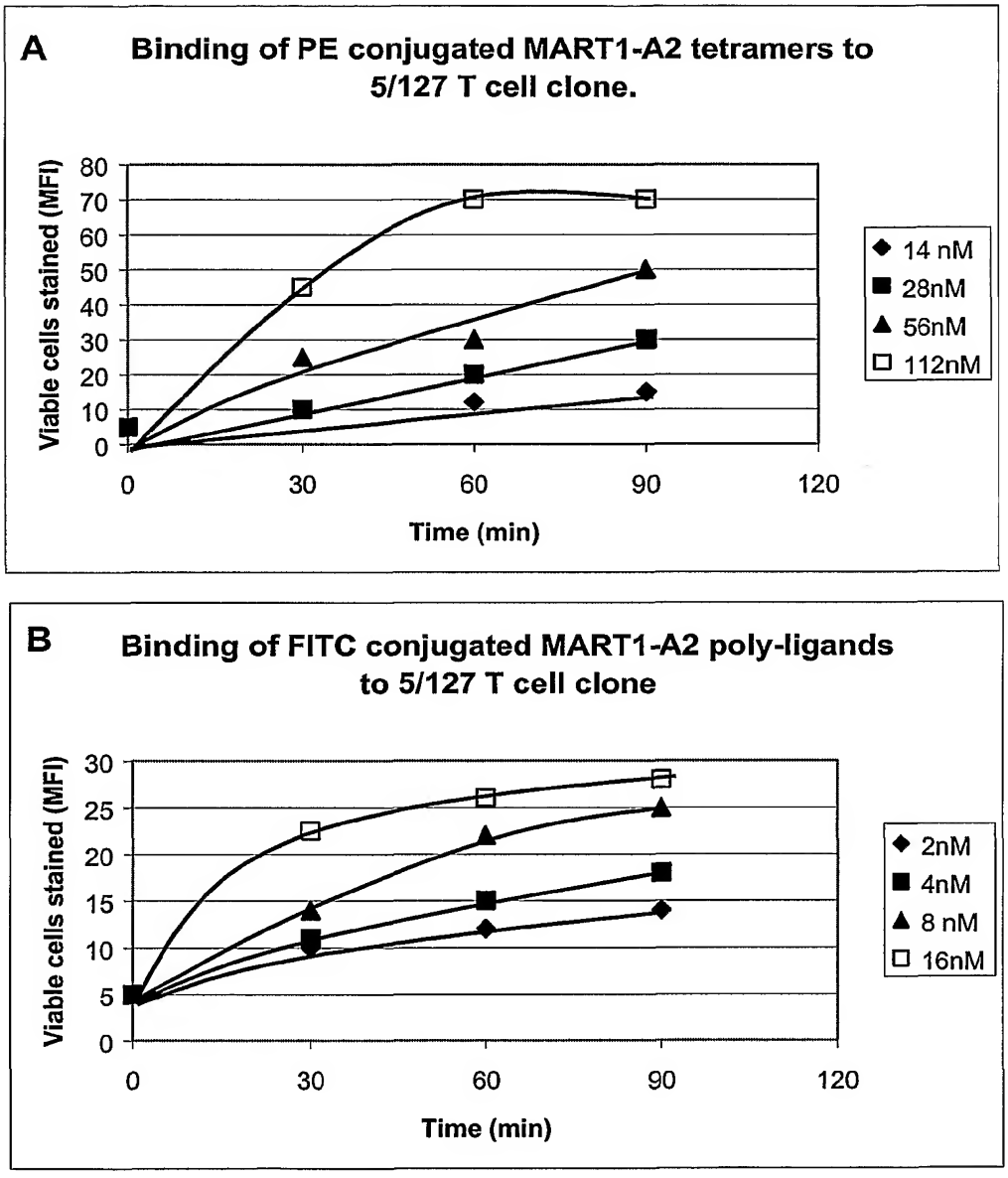

A
Binding of PE conjugated MART1-A2 tetramers to 5/127 T cell clone.
Viable cells stained (MFI)
80
70
60
50
40
30
20
10
0
0
30
60
90
120
Time (min)
14 nM
28nM
56nM
112nM
B
Binding of FITC conjugated MART1-A2 poly-ligands to 5/127 T cell clone
Viable cells stained (MFI)
30
25
20
15
10
5
0
0
30
60
90
120
Time (min)
2nM
4nM
8 nM
16nM

Figure 27

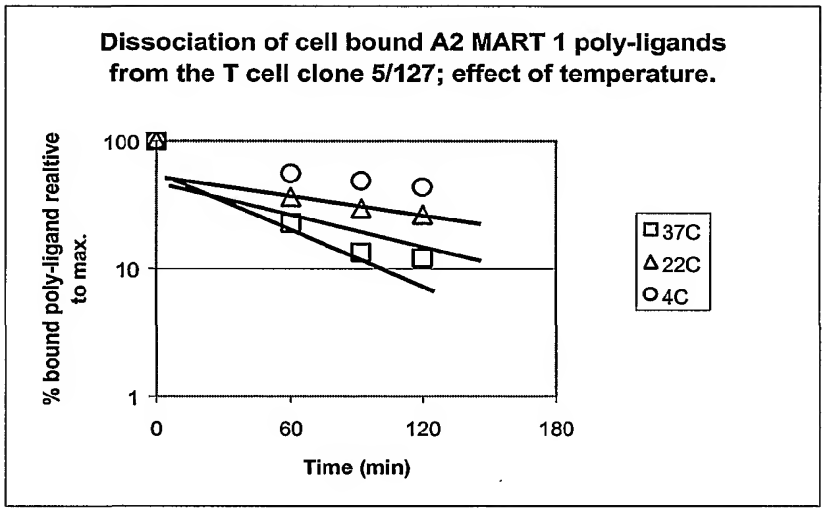
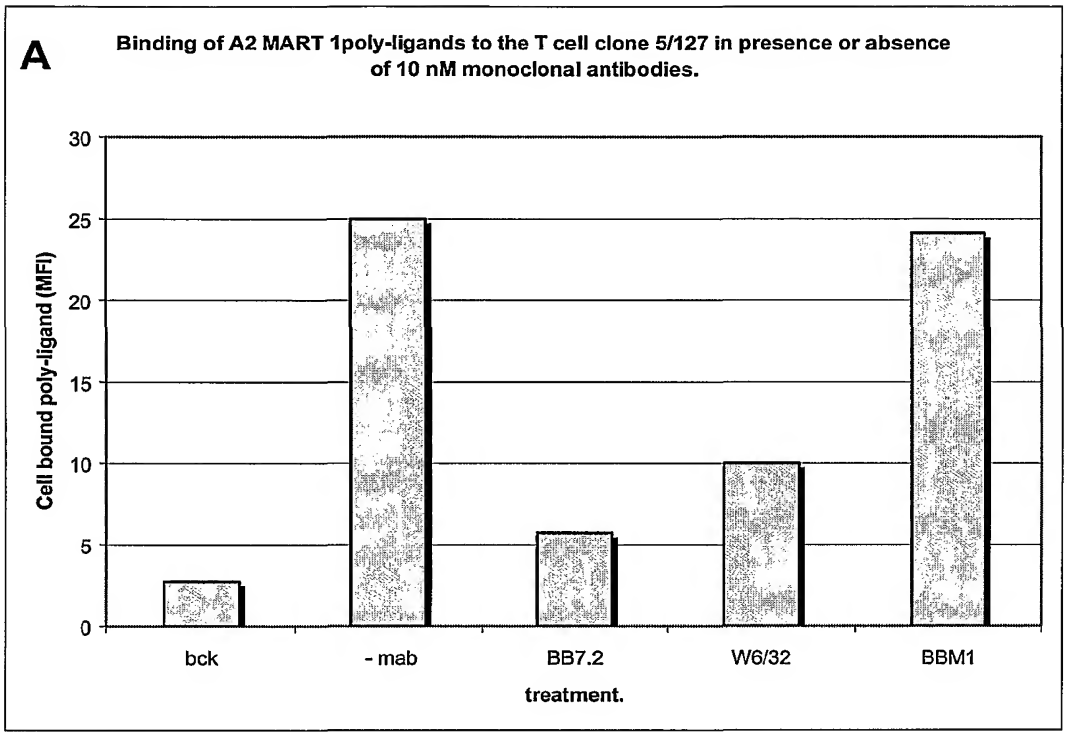

Dissociation of cell bound A2 MART 1 poly-ligands from the T cell clone 5/127; effect of temperature.
% bound poly-ligand realtive to max.
100
10
1
0
60
120
180
Time (min)
37C
22C
4C

Figure 28


A
Binding of A2 MART 1poly-ligands to the T cell clone 5/127 in presence or absence of 10 nM monoclonal antibodies.
Cell bound poly-ligand (MFI)
30
25
20
15
10
5
0
bck
- mab
BB7.2
W6/32
BBM1
treatment.

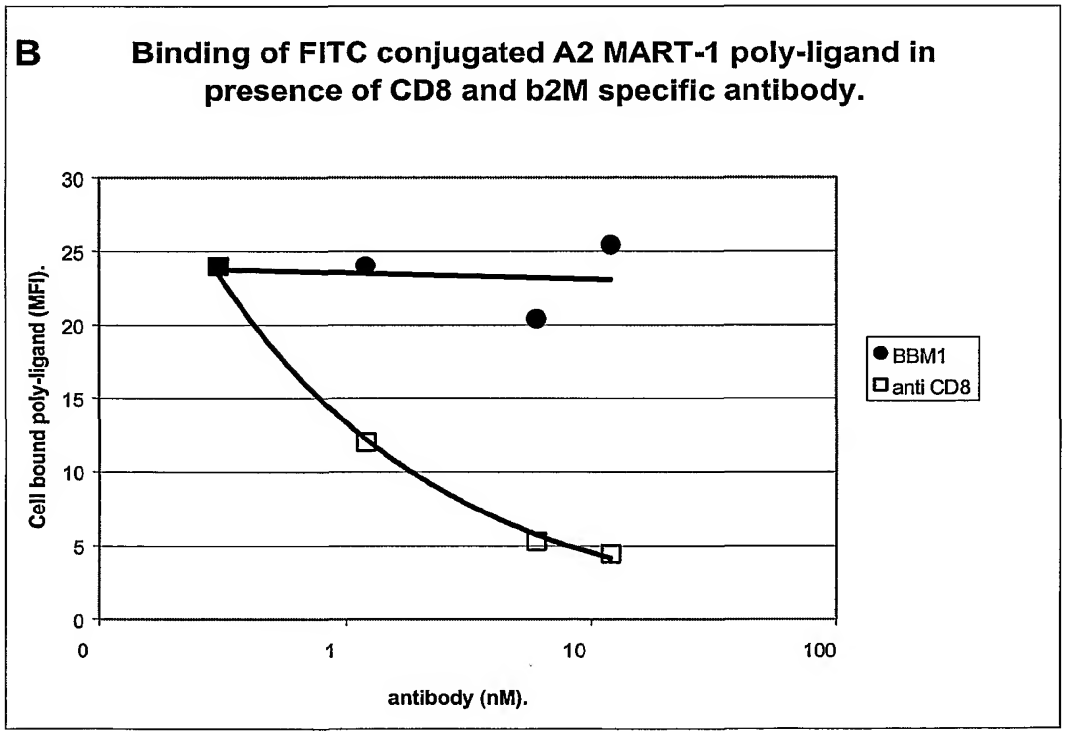

B
Binding of FITC conjugated A2 MART-1 poly-ligand in presence of CD8 and b2M specific antibody.
Cell bound poly-ligand (MFI).
30
25
20
15
10
5
0
0
1
10
100
antibody (nM).
BBM1
anti CD8

Figure 29

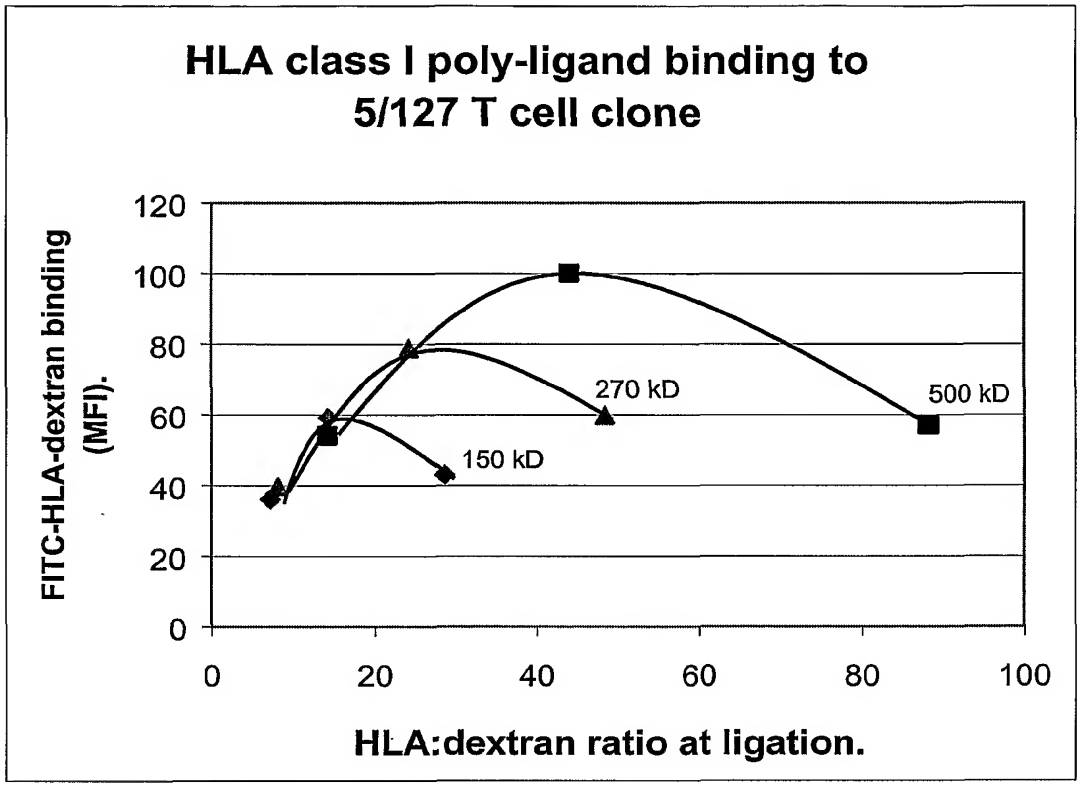
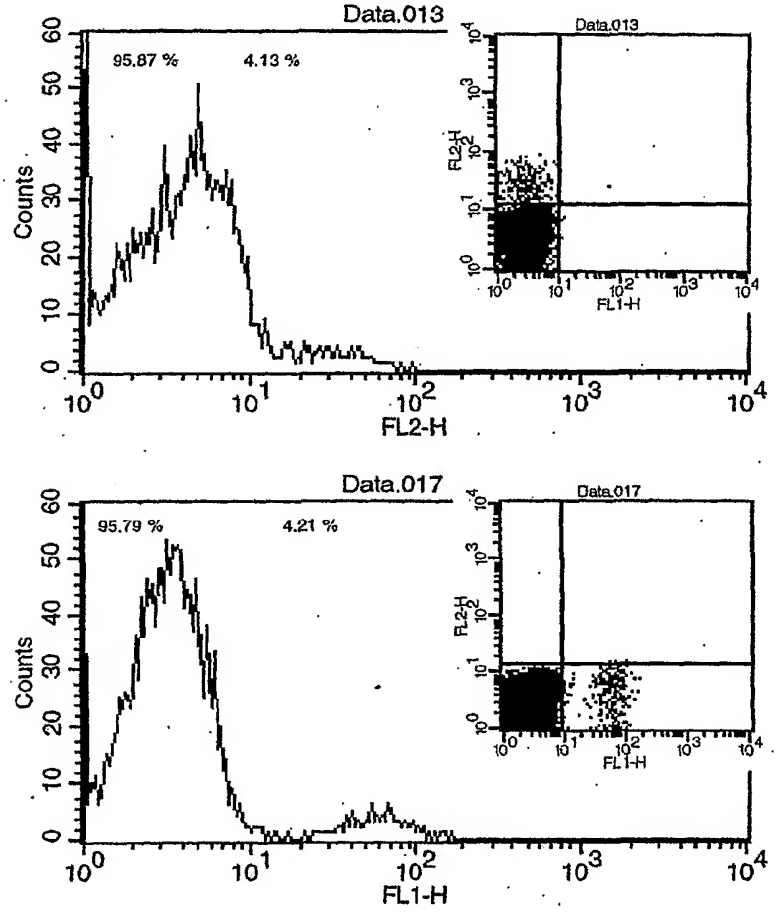

HLA class I poly-ligand binding to 5/127 T cell clone
FITC-HLA-dextran binding (MFI).
120
100
80
60
40
20
0
0
20
40
60
80
100
270 kD
500 kD
150 kD
HLA:dextran ratio at ligation.

Figure 30

Flow cytometry analysis of Mart-1 specific T cell subpopulation



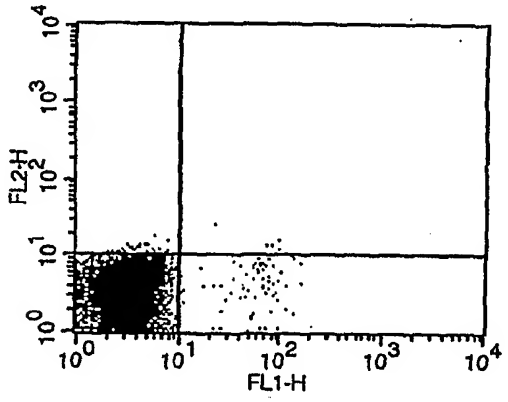
A: Staining with tetramer Mart-1/HLA protein (15 nM) (PE conjugated).

B: Staining with poly-ligand Mart-1/HLA protein (3 nM) (FITC conjugated).

Figure 31

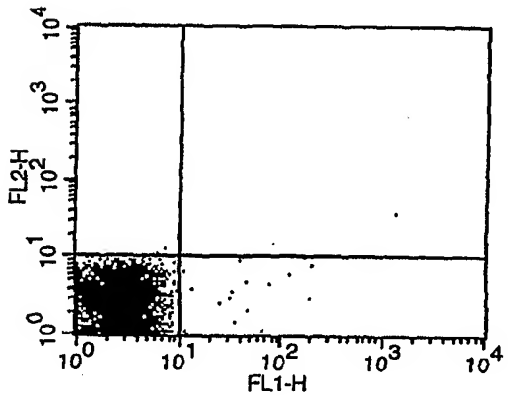
Flow cytometry analysis of Mart-1 (clone 5/127) and gp100 (clone 5/130) specific T cells.

A



poly-ligand Mart-1/HLA A0201 protein

B



poly-ligand gp100/HLA A0201 protein

Figure 32

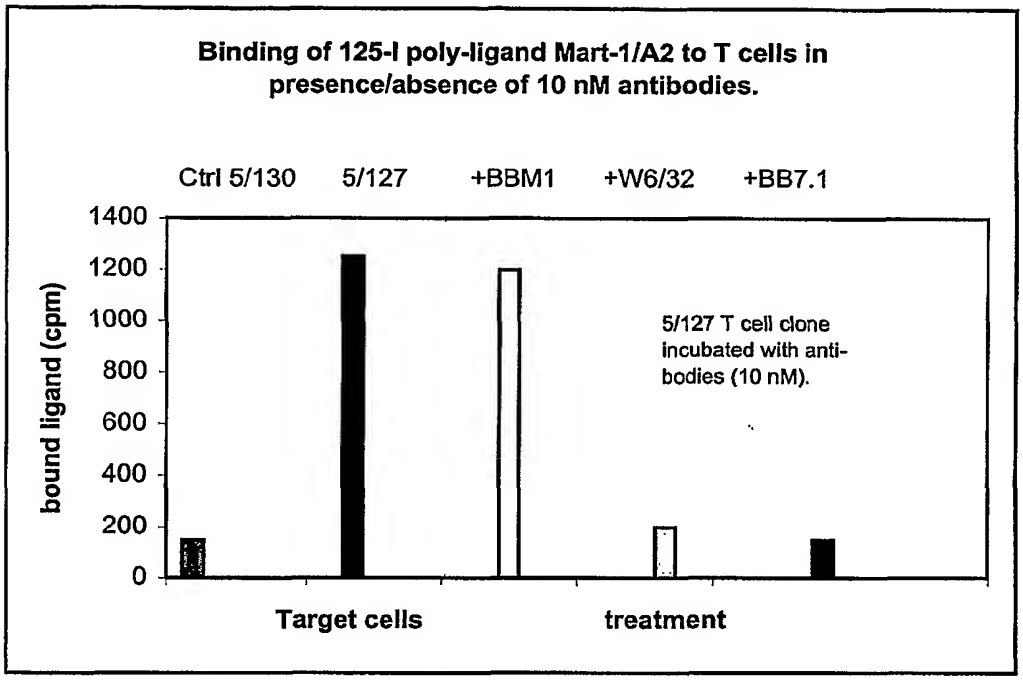

Binding of 125-I poly-ligand Mart-1/A2 to T cells in presence/absence of 10 nM antibodies.
Ctrl 5/130
5/127
+BBM1
+W6/32
+BB7.1
1400
1200
1000
800
600
400
200
0
bound ligand (cpm)
5/127 T cell clone incubated with anti-bodies (10 nM).
Target cells
treatment

Figure 33

Figure 34

In the table, "X" indicates variable amino acids.

Interesting HLA Class I and II Binding Motif List				
#	Class	Motif	Serotype	Genotype
1	Class I	XX[DE]XXXXXX[Y]	A1	
2	Class I	XX[DE]XXXXX[Y]	A1	
3	Class I	XX[DE]XXXX[Y]	A1	
4	Class I	XXXXXXXXX[K]	A11	A*1101
5	Class I	XXXXXXX[K]	A11	A*1101
6	Class I	XXXXXXXX[K]	A11	A*1101
7	Class I	X[LM]XXXXXX[VL]	A2	A*0201
8	Class I	X[LM]XXXXX[VL]	A2	A*0201
9	Class I	X[LM]XXXXXX[VL]	A2	A*0201
10	Class I	X[L]XXXXXX[LV]	A2	A*0202
11	Class I	X[L]XXXXXXX[LV]	A2	A*0202
12	Class I	X[L]XXXXX[LV]	A2	A*0202
13	Class I	X[L]XXXXXXX[L]	A2	A*0204
14	Class I	X[L]XXXXX[L]	A2	A*0204
15	Class I	X[L]XXXXXX[L]	A2	A*0204
16	Class I	X[VLIMQ]XXXXXX[L]	A2	A*0205
17	Class I	X[VLIMQ]XXXXXXX[L]	A2	A*0205
18	Class I	X[VLIMQ]XXXXX[L]	A2	A*0205
19	Class I	X[V]XXXXXXX[V]	A2	A*0206
20	Class I	X[V]XXXXX[V]	A2	A*0206
21	Class I	X[V]XXXXXX[V]	A2	A*0206
22	Class I	X[L][D]XXXXXX[L]	A2	A*0207
23	Class I	X[L][D]XXXX[L]	A2	A*0207

24	Class I	X[L][D]XXXXX[L]	A2	A*0207
25	Class I	X[VQL]XXXXXX[LV]	A2	A*0214
26	Class I	X[VQL]XXXXXXX[LV]	A2	A*0214
27	Class I	X[VQL]XXXXX[LV]	A2	A*0214
28	Class I	X[Y]XXXXXXX[ILF]	A24	
29	Class I	X[Y]XXXXXX[ILF]	A24	
30	Class I	X[Y]XXXXX[ILF]	A24	
31	Class I	X[E]XXXXXXX[Y]	A29	A*2902
32	Class I	X[E]XXXXX[Y]	A29	A*2902
33	Class I	X[E]XXXXXX[Y]	A29	A*2902
34	Class I	X[LVM]XXXXXXX[KYF]	A3	
35	Class I	X[LVM]XXXXX[KYF]	A3	
36	Class I	X[LVM]XXXXXX[KYF]	A3	
37	Class I	XXXXXXXX[R]	A31	A*3101
38	Class I	XXXXXXXXX[R]	A31	A*3101
39	Class I	XXXXXXX[R]	A31	A*3101
40	Class I	XXXXXXXXX[R]	A33	A*3302
41	Class I	XXXXXXXX[R]	A33	A*3302
42	Class I	XXXXXXX[R]	A33	A*3302
43	Class I	X[VT]XXXXX[RK]	A68	A*6801
44	Class I	X[VT]XXXXXX[RK]	A68	A*6801
45	Class I	X[VT]XXXXXX[RK]	A68	A*6801
46	Class I	X[VTA]XXXXXX[VL]	A69	A*6901
47	Class I	X[VTA]XXXXXXX[VL]	A69	A*6901
48	Class I	X[VTA]XXXXX[VL]	A69	A*6901
49	Class I	X[RK]XX[RH]XXX[L]	B14	
50	Class I	X[RK]XX[RH]XX[L]	B14	
51	Class I	X[RK]XX[RH]XXXX[L]	B14	
52	Class I	X[R]XXXXXXXX	B27	

53	Class I	X[R]XXXXXX	B27	
54	Class I	X[R]XXXXXXX	B27	
55	Class I	X[R]XXXXXX[FYILW]	B27	B*2702
56	Class I	X[R]XXXXX[FYILW]	B27	B*2702
57	Class I	X[R]XXXXXXX[FYILW]	B27	B*2702
58	Class I	X[R]XXXXXXX[LF]	B27	B*2705
59	Class I	X[R]XXXXXX[LF]	B27	B*2705
60	Class I	X[R]XXXXX[LF]	B27	B*2705
61	Class I	X[P]XXXXXXX[YFMLI]	B35	B*35
62	Class I	X[P]XXXXXX[YFMLI]	B35	B*35
63	Class I	X[P]XXXXX[YFMLI]	B35	B*35
64	Class I	X[P]XXXXXXX[YFMLI]	B35	B*3501
65	Class I	X[P]XXXXX[YFMLI]	B35	B*3501
66	Class I	X[P]XXXXXX[YFMLI]	B35	B*3501
67	Class I	X[P]XXXXX[M]	B35	B*3503
68	Class I	X[P]XXXXXXX[M]	B35	B*3503
69	Class I	X[P]XXXXXX[M]	B35	B*3503
70	Class I	X[DE]XXXXX[FML][IL]	B37	B*3701
71	Class I	X[DE]XXXXXX[FML][IL]	B37	B*3701
72	Class I	X[DE]XXXX[FML][IL]	B37	B*3701
73	Class I	XXXXXXXXX[FL]	B38	B*3801
74	Class I	XXXXXXXX[FL]	B38	B*3801
75	Class I	XXXXXXX[FL]	B38	B*3801
76	Class I	X[RH]XXXXXXX[L]	B39	B*39011
77	Class I	X[RH]XXXXXX[L]	B39	B*39011
78	Class I	X[RH]XXXXX[L]	B39	B*39011
79	Class I	X[KQ]XXXXXX[L]	B39	B*3902
80	Class I	X[KQ]XXXXXXX[L]	B39	B*3902
81	Class I	X[KQ]XXXXX[L]	B39	B*3902

82	Class I	X[E]XXXXXXX[L]	B40	
83	Class I	X[E]XXXXX[L]	B40	
84	Class I	X[E]XXXXXX[L]	B40	
85	Class I	X[E]XXXXXXX[Y]	B44	
86	Class I	X[E]XXXXX[Y]	B44	
87	Class I	X[E]XXXXXX[Y]	B44	
88	Class I	X[E]XXXXX[FY]	B44	B*4402
89	Class I	X[E]XXXXXXX[FY]	B44	B*4402
90	Class I	X[E]XXXXXX[FY]	B44	B*4402
91	Class I	X[E]XXXXX[YF]	B44	B*4403
92	Class I	X[E]XXXXXXX[YF]	B44	B*4403
93	Class I	X[E]XXXXXX[YF]	B44	B*4403
94	Class I	X[M]XXXXXXX[YF]	B46	B*4601
95	Class I	X[M]XXXXXX[YF]	B46	B*4601
96	Class I	X[M]XXXXX[YF]	B46	B*4601
97	Class I	X[APG]XXXXX[FI]	B51	B*5101
98	Class I	X[APG]XXXXXX[FI]	B51	B*5101
99	Class I	X[APG]XXXXXXX[FI]	B51	B*5101
100	Class I	X[PAG]XXXXXX[IV]	B51	B*5102
101	Class I	X[PAG]XXXXXXX[IV]	B51	B*5102
102	Class I	X[PAG]XXXXX[IV]	B51	B*5102
103	Class I	X[APG]XXXXXX[VIF]	B51	B*5103
104	Class I	X[APG]XXXXX[VIF]	B51	B*5103
105	Class I	X[APG]XXXXXXX[VIF]	B51	B*5103
106	Class I	XXXXXX[IV][IV]	B52	B*5201
107	Class I	XXXXXXX[IV][IV]	B52	B*5201
108	Class I	XXXXXXXX[IV][IV]	B52	B*5201
109	Class I	X[P]XXXXXX[LIVMY]	B53	B*5301
110	Class I	X[P]XXXXX[LIVMY]	B53	B*5301

111	Class I	X[P]XXXXXXX[LIVMY]	B53	B*5301
112	Class I	X[P]XXXXXXX	B54	B*5401
113	Class I	X[P]XXXXXXXX	B54	B*5401
114	Class I	X[P]XXXXXX	B54	B*5401
115	Class I	X[P]XXXXXXX	B55	B*5501
116	Class I	X[P]XXXXXXXX	B55	B*5501
117	Class I	X[P]XXXXXX	B55	B*5501
118	Class I	X[P]XXXXXXX	B55	B*5502
119	Class I	X[P]XXXXXX	B55	B*5502
120	Class I	X[P]XXXXXXXX	B55	B*5502
121	Class I	X[P]XXXXXXX[A]	B56	B*5601
122	Class I	X[P]XXXXXX[A]	B56	B*5601
123	Class I	X[P]XXXXX[A]	B56	B*5601
124	Class I	X[AST]XXXXXXX[FW]	B58	B*5801
125	Class I	X[AST]XXXXX[FW]	B58	B*5801
126	Class I	X[AST]XXXXXX[FW]	B58	B*5801
127	Class I	X[E]XXXXXX[L]	B60	B*40012
128	Class I	X[E]XXXXXXX[L]	B60	B*40012
129	Class I	X[E]XXXXX[L]	B60	B*40012
130	Class I	X[E]XXXXXX[V]	B61	B*4006
131	Class I	X[E]XXXXXXX[V]	B61	B*4006
132	Class I	X[E]XXXXX[V]	B61	B*4006
133	Class I	X[QL]XXXXXX[FY]	B62	B*1501
134	Class I	X[QL]XXXXX[FY]	B62	B*1501
135	Class I	X[QL]XXXXXXX[FY]	B62	B*1501
136	Class I	X[P]XXXXXXX[L]	B67	B*6701
137	Class I	X[P]XXXXXX[L]	B67	B*6701
138	Class I	X[P]XXXXX[L]	B67	B*6701
139	Class I	X[P]XXXXXX[LF]	B7	

140	Class I	X[P]XXXXX[LF]	B7	
141	Class I	X[P]XXXXXXX[LF]	B7	
142	Class I	X[P]XXXXXX[L]	B7	B*0702
143	Class I	X[P]XXXXX[L]	B7	B*0702
144	Class I	X[P]XXXXXXX[L]	B7	B*0702
145	Class I	X[P]XXXXXXX[L]	B7	B*0703
146	Class I	X[P]XXXXX[L]	B7	B*0703
147	Class I	X[P]XXXXXX[L]	B7	B*0703
148	Class I	X[P]XXXXXXX[L]	B7	B*0705
149	Class I	X[P]XXXXXX[L]	B7	B*0705
150	Class I	X[P]XXXXX[L]	B7	B*0705
151	Class I	X[R]XXXXX[P]	B73	B*7301
152	Class I	X[R]XXXXXX[P]	B73	B*7301
153	Class I	X[R]XXXXXXX[P]	B73	B*7301
154	Class I	X[PAG]XXXXXXX	B78	B*7801
155	Class I	X[PAG]XXXXXX	B78	B*7801
156	Class I	X[PAG]XXXXXXXX	B78	B*7801
157	Class I	XX[K]X[KR]XX[L]	B8	
158	Class I	XX[K]X[KR]XXXX[L]	B8	
159	Class I	XX[K]X[KR]XXX[L]	B8	
160	Class I	X[YPF]XXXXXXX[LF]	C4	Cw*0401
161	Class I	X[YPF]XXXXXX[LF]	C4	Cw*0401
162	Class I	X[YPF]XXXXX[LF]	C4	Cw*0401
163	Class I	X[AL]XXXXXX[L]	Cw1	Cw*0102
164	Class I	X[AL]XXXXXXX[L]	Cw1	Cw*0102
165	Class I	X[AL]XXXXX[L]	Cw1	Cw*0102
166	Class I	X[A]XXXXXXX[LM]	Cw10	Cw*0304
167	Class I	X[A]XXXXX[LM]	Cw10	Cw*0304
168	Class I	X[A]XXXXXX[LM]	Cw10	Cw*0304

169	Class I	XXXXXXXXX[LFMI]	Cw3	Cw*0301
170	Class I	XXXXXXX[LFMI]	Cw3	Cw*0301
171	Class I	XXXXXXXX[LFMI]	Cw3	Cw*0301
172	Class I	XXXXXXX[LIVF]	Cw6	Cw*0601
173	Class I	XXXXXXXXX[LIVF]	Cw6	Cw*0601
174	Class I	XXXXXXXX[LIVF]	Cw6	Cw*0601
175	Class I	XXXXXXXX[LIVF]	Cw6	Cw*0602
176	Class I	XXXXXXX[LIVF]	Cw6	Cw*0602
177	Class I	XXXXXXXXX[LIVF]	Cw6	Cw*0602
178	Class I	XXXXXXX[YFL]	Cw7	Cw*0702
179	Class I	XXXXXXXXX[YFL]	Cw7	Cw*0702
180	Class I	XXXXXXXX[YFL]	Cw7	Cw*0702
181	Class II	[MV]XXX[MY]XX[MV]X	DPA1*0102	DPA1*0102
182	Class II	[WY]XXXXXXXX	DPA1*0102	DPA1*0102
183	Class II	[FL]XXX[FL]XX[IA]X	DPA1*0102	DPA1*0102
184	Class II	XXXXXXXXX[L]	DPA1*0201	DPA1*0201
185	Class II	[A]XXXXX[A]XXX	DPA1*0201	DPA1*0201
186	Class II	[WY]XXXXXXXX	DPB1*0201	DPB1*0201
187	Class II	[MV]XXX[MY]XX[MV]X	DPB1*0201	DPB1*0201
188	Class II	[FL]XXX[FL]XX[IA]X	DPB1*0201	DPB1*0201
189	Class II	[A]XXXXX[A]XXX	DPw4	DPB1*0401
190	Class II	XXXXXXXXX[L]	DPw4	DPB1*0401
191	Class II	XXX[I]XXXXX	DQ2	DQB1*0201
192	Class II	XXXXXXXX[M]	DQ2	DQB1*0201
193	Class II	XXXXX[E]XXX	DQ2	DQB1*0201
194	Class II	XXXX[Y]XXXX	DQ7	DQB1*0301
195	Class II	XXXXXXXX[M]	DQA1*0501	DQA1*0501
196	Class II	XXX[I]XXXXX	DQA1*0501	DQA1*0501
197	Class II	XXXX[Y]XXXX	DQA1*0501	DQA1*0501

198	Class II	XXXXX[E]XXX	DQA1*0501	DQA1*0501
199	Class II	XXXXX[P]XXX	DR1	DRB1*0101
200	Class II	XXX[Q]XXXX[Y]	DR1	DRB1*0101
201	Class II	XXXXX[P]XXX	DR1	DRB1*0102
202	Class II	XXX[M]XXXXX	DR1	DRB1*0102
203	Class II	[F]XXXX[H]XXX	DR11	DRB1*1101
204	Class II	[V]XXXXXXXX	DR11	DRB1*1104
205	Class II	[L]XX[M]XXXXX	DR11	DRB1*1104
206	Class II	[I]XXXX[R]XXX	DR11	DRB1*1104
207	Class II	[V]X[A]XXXXX[V]	DR12	DRB1*1201
208	Class II	[IL]XX[L]X[R]XX[Y]	DR13	DRB1*1301
209	Class II	[V]XX[V]X[K]XX[F]	DR13	DRB1*1301
210	Class II	XXX[M]XXXX[A]	DR13	DRB1*1301
211	Class II	XXX[A]XXXX[S]	DR13	DRB1*1301
212	Class II	XXX[W]XXXX[T]	DR13	DRB1*1301
213	Class II	XXX[Y]XXXXX	DR13	DRB1*1301
214	Class II	XXXXX[R]XXX	DR13	DRB1*1302
215	Class II	[I]XX[W]XXXXX	DR13	DRB1*1302
216	Class II	[A]XX[A]XXXX[T]	DR13	DRB1*1302
217	Class II	[V]XX[M]X[K]XXX	DR13	DRB1*1302
218	Class II	XXX[Y]XXXXX	DR13	DRB1*1302
219	Class II	[I]XX[I]XX[V]XX	DR15	DRB1*1501
220	Class II	[L]XX[F]XX[I]XX	DR15	DRB1*1501
221	Class II	XXXXXX[F]XX	DR15	DRB1*1501
222	Class II	[V]XX[Y]XX[L]XX	DR15	DRB1*1501
223	Class II	XXXXXX[M]XX	DR15	DRB1*1501
224	Class II	XXXXXXXX[F]	DR17	DRB1*0301
225	Class II	[V]XXXX[N]XXX	DR17	DRB1*0301
226	Class II	XXX[D]XXXXX	DR17	DRB1*0301

227	Class II	[M]XXXXXXXX	DR4	DRB1*0401
228	Class II	XXXXXXXX[K]	DR4	DRB1*0401
229	Class II	XXX[M]XXXXX	DR4	DRB1*0402
230	Class II	XXXXX[K]XX[H]	DR4	DRB1*0402
231	Class II	XXX[W]XXXXX	DR4	DRB1*0402
232	Class II	XXXXX[R]XX[K]	DR4	DRB1*0404
233	Class II	[M]XXXXXXXX	DR4	DRB1*0405
234	Class II	[W]XX[VK]X[DS]XX[N]	DR4	DRB1*0407
235	Class II	[FY]XX[A]X[NT]XX[Q]	DR4	DRB1*0407
236	Class II	XXX[N]XXXXX	DR52	DRB3*0202
237	Class II	XXXXXXXX[V]	DR52	DRB3*0301
238	Class II	[IL]XX[N]X[AS]XX[IL]	DR52	DRB3*0301
239	Class II	XXX[S]XXXXX	DR9	DRB1*0901

Figure 35

HIV/SIV proteins (source http://hiv-web.lanl.gov/immunology/-index.html)

Name	Size	Function
Gag MA	p17	membrane anchoring, env interaction
CA	p24	core capsid virion
NC	p7	nucleocapsid, binds RNA virion Vpr virion
Protease (RP)	p15	gag/pol cleavage and maturation virion
Reverse trans-scriptase (RT)	p66	p51 reverse transcription virion
Env	pg120/gp41	external viral glycoproteins bind to CD4 and chemokine co-receptors plasma membrane
Tat	p16/p14	viral transcriptional transactivator
Rev	p19	RNA transport, stability and utilisation fact
Nef	p27-p25	CD4 and MHC Class I downregilation
Vpx	p12-16	Vpr homolog? Present in HIV-2
Tev	p28	Tripartite tat-env-rev protein

Figure 36

Tumour-associated antigens recognised by T-lymphocytes (source: Danish Cancer Society)

Proteins over-expressed in tumours

p53, WT1, HER-2/neu, alpha fetoprotein, MUC-1, MUC-2, telomerase, survinin, FBP

Virial proteins

HPV proteins (E6 and E7), EBV (LPM2)

Mutated, unique or aberrantly expressed proteins

CDK-4, p21 ras, MUM-1, MUM-2, MUM-3 (helicase), beta-catenin, NA17-A/Gnt-V, p15, pg100-in4, caspase-8, hsp70-2, elongation factor 2, mutated HLA-A2, class I myosin, intestinal carboxyl esterase, BCR-ABL fusion protein, idiotype

Lineage-specific differentiation antigens

Prostate: PSA, PSMA, PAP

Melanoma: tyrosinase, gp100, MART-1, TRP-1, TRP-2, MC1R

Cancer/testis antigens

MAGE-A family: MAGE-A1, MAGE-A2, MAGE-A3, MAGE-A4, MAGE-A6, MAGE-A10, MAGE-A12

MAGE-B family: MAGE-B1, MAGE-B2, MAGE-B6

GAGE family: GAGE-1, GAGE-2, GAGE-3, GAGE-4, GAGE-5, GAGE-6, GAGE-7B, GAGE-8

LAGE family: LAGE-1a, LAGE-1b, NY-ESO-1

Separate members: BAGE, PRAME (MAPE), SART-1, SART-3, ART-4

Figure 37

Best defined HIV CTL epitopes (sourse **http://hiv-web.lanl.gov/immunology/index.html**)

HLA	Protein	AA	Isolate	Sequence
HLA-A2				
	p17	77-85	LAI	SLYNTVATL
	Johnson91			
	Parker92			
	Parker94			
	RT	33-41	LAI	ALVEICTEM
	RT	346-354	LAI	VIYQYMDDL
	Walker89			
	Tsomides91			
A*0201				
	pg120	311-320	III-B	RGPGRAFVTI
	gp41	818-827	LAI	SLLNATDIAV
	nef	136-145	LAI	PLTFGWCYKL
	nef	180-189	LAI	VLEWRFDSRL
HLA-A3.1				
	p17	18-26	LAI	KIRLRPGGK
	p17	20-28	LAI	RLRPGGKKK
	p17	20-29	LAI	RLRPGGKKKY
	RT	33-43	LAI	ALVEICTEMEK
	RT	325-333	LAI	AIFQSSMTK
	gp120	37-46	LAI	TVYYGVPVWK
	gp41	775-785	LAI	RLRDLLLIVTR
	nef	73-82	LAI	QVPLRPMTYK
HLA-A11				
	p17	84-92	LAI	TLYVCHQRI
	p24	349-359	III-B	ACQGVGGPGGHK
	RT	325-333	LAI	AIFQSSMTK
	RT	508-517	LAI	PLRPMTYK

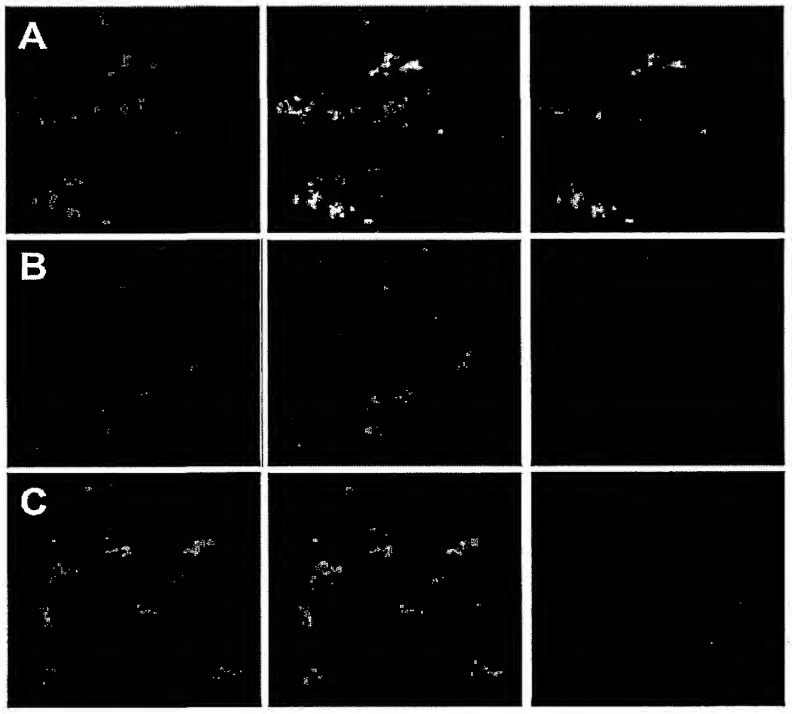
	nef	75-82	LAI	PLRPMTYK
	nef	84-82	LAI	AVDLSHFLK
HLA-A19				
A*7401	RT	71-79	Clade A/B/D	ITLWQRPLV
HLA-A24				
	p17	28-36	LAI	KYKLKHIVW
	p24	296-306	HIL-1 Clade A	RDYVDRFFKTL
	gp120	53-62	LAI	LFCASDAKAY
	gp41	591-598	LAI	YLKDQQLL
	nef	138-147	LAI	RYPLTFGW
HLA-A25				
	p24	145-155	LAI	QAISPRTLNAW
	p24	203-212	LAI	ETINEEAAEW
HLA-A26				
	p24	167-175	LAI	EVIPMFSAL
	RT	593-603	LAI	ETFYVDGAANR
HLA-A28				
A*6802	RT	71-79	Clade A/B/D	ITLWQRPLV
A*6802	RT	85-93	Clade D	DTVLEEMNL
HLA-A29				
	gp120	376-384	LAI	FNCGGEFFY
HLA-A31				
	gp41	775-785	LAI	RLRDLLLIVTR
HLA-A32				
	RT	559-568	LAI	PIQKETWETW
	gp120	419-427	HXB2	RIKQIINMW
HLA-B7				
	p24	148-156	LAI	SPRTLNAWV
	p24	179-187	LAI	ATPQDLNTM
	RT	323-332	LAI	SPAIFQSSMT

	gp120	303-312	LAI	RPNNNTRKSI
	gp41	843-851	LAI	IPRRIRQGL
	nef	68-77	LAI	FPVTPQVPLR
	nef	77-85	LAI	RPMTYKAAL
	nef	128-137	LAI	TPGPGVRYPL
HLA-B8				
	p17	24-31	LAI	GGKKKYKL
	p17	74-82	lai	ELRSLYNTV
	p24	260-267	LAI	EIYKRWII
	gp120	2-10	III-B	RVKEKYQHL
	gp41	591-598	LAI	YLKDQQLL
	Johnson92			
	Shankar96			
	nef	13-20	LAI	WPTVRERM
	nef	90-97	LAI	FLKEKGGL
	Culmann-Peciolelli94			
	Price97			
HLA-B14				
	p24	298-306	LAI	DRFYKTLRA
	gp41	589-597	LAI	ERYLKDQQL
HLA-B15				
	gp120	375-383	LAI	SFNCGGEFF
HLA-B18				
	p24	293-302	HIV-1 Clade B/D	FRDYVDRFYK
	nef	135-143	LAI	YPLTFGWCY
HLA-B27				
	p17	18-27	LAI	KIRLRPGGKK
	p17	19-27	LAI	IRLRPGGKK
	p24	263-272	LAI	KRWIILGLNK
	Nixon88, Buseyne93			
	gp41	590-597	LAI	RYLKDQQL

	gp41	791-799	LAI	GRRGWEALKY
HLA-B*2703				
	p24	265-274	HIV-2	RRWIQLGLQK
B*2705	nef	105-114	LAI	RRQDILDLWI
	nef	73-82	LAI	QVPLRPMTYK
	nef	134-141	LAI	RYPLTFGW
HLA-B35				
	p17	124-132	JH31	NSSKVSQNY
	p17	36-44	LAI	WASRELERF
	p24	254-262	U455	PPIPVGDIY
	RT	262-270	LAI	TVLDVGDAY
	RT	273-282	III-B	VPLDEDFRKY
	Sipsas97, Shiga96			
	RT	328-336	III-B	HPDIVIYQY
	gp120	42-52	LAI	VPVWKEATTTL
	gp41	611-619	LAI	TAVPWNASW
	nef	74-81	LAI	VPLRPMTY
	Culmann91, Culmann-Penciolelli94			
	gag	245-253	HIV-2	NPVPVGNIY
HLA-B37				
	nef	120-128	LAI	YFPDWQNYT
HLA-B29				
	p24	193-201	LAI	GHQAAMQML
HLA-B42				
	p17	20-29	LAI	RLRPGGKKY
	RT	438-446	LAI	YPGIKVRQL
HLA-B44				
	p17	306-316	SF2	AEQASQDVKNW
	gp120	30-38	SF33	AENLWVTVY
HLA-B*4402				

	p24	294-304	HIV-1 Clade B	RDYVDRFYKTL
HLA-B45				
	RT	591-600	LAI	GAETFYVDGA
HLA-B51				
	p24	325-333	LAI	NANPDCKTI
	RT	42-50	LAI	EKEGKISKI
	RT	295-302	III-B	TAFTIPSI
	gp41	557-565	III-B	RAIEAQQHL
HLA-B52				
	p24	275-282	LAI	RMYSPTSI
HLA-B53				
	HIV-2 gag	173-181	HIV-2	TPYDINQML
HLA-B55				
	gp120	42-51	LAI	VPVWKEATTT
HLA-B57				
	p24	147-1555	III-B	ISPRTLNAW
	Johnson91, Goulder96			
	p24	140-149	LAI	TSTLQEQIGW
	p24	162-172	LAI	KAFSPEVIPMF
	p24	240-249	LAI	TSTLQEQIGW
	p24	311-319	LAI	QASQEVKNW
	p24	311-319	LAI	QASQDVKNW
	nef	116-125	LAI	HTQGYFPDWQ
	nef	120-128	LAI	YFPDWQNYT
HLA-B58				
	p24	240-249	LAI	TSTLQEQIGW
HLA-B60				
	p17	240-249	LAI	TSTLQEQIGW
HLA-Bw62				
	p17	20-29	LAI	RLRPGGKKKY
	p24	268-277	LAI	LGLNKIVRMY

	RT	415-426	III-B	LVGKLNWASQIY
	RT	476-485	LAI	ILKEPVHGVY
	nef	84-91	LAI	AVDLSHFL
	Culmann-Peniolelli94			
	nef	117-127	LAI	TQGYFPDWQNY
HLA-Cw*01,02				
	p24	168-175	LAI	VIPMFSAL
HLA-Cw4				
	gp120	380-388	LAI	SFNCGGEFF



(A) CD8 Cy3
A*0201/Sur1M2 FITC
HLA A2+

(B) CD8 Cy3
A*0201/gp100 FITC
HLA A2+

(C) CD8 Cy3
A*0201/Sur1M2 FITC
HLA A2-

Figure 38

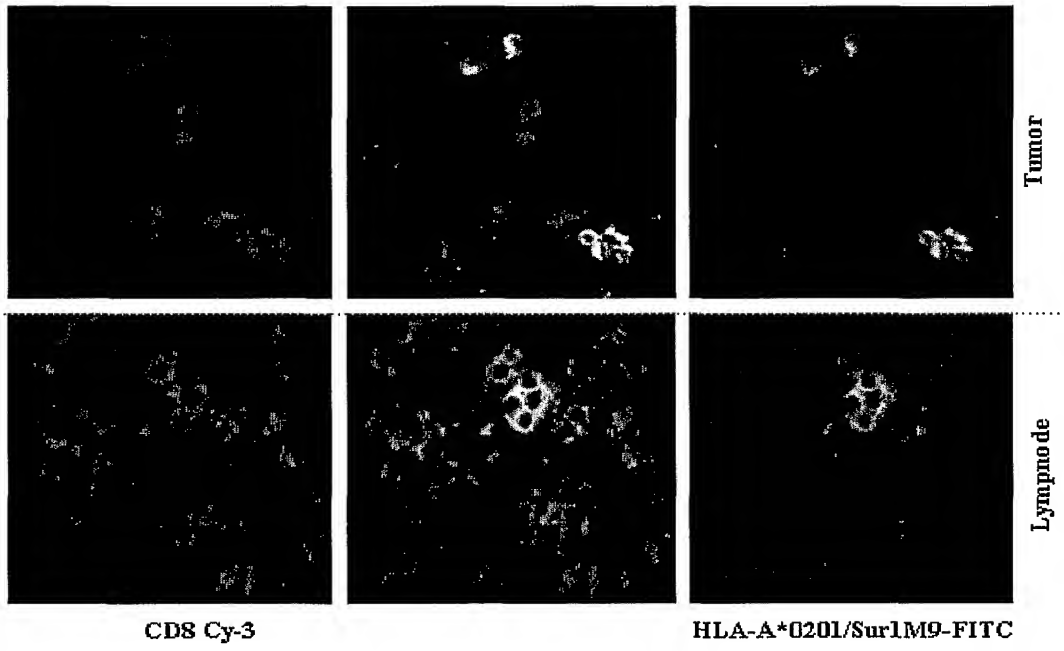


Figure 39

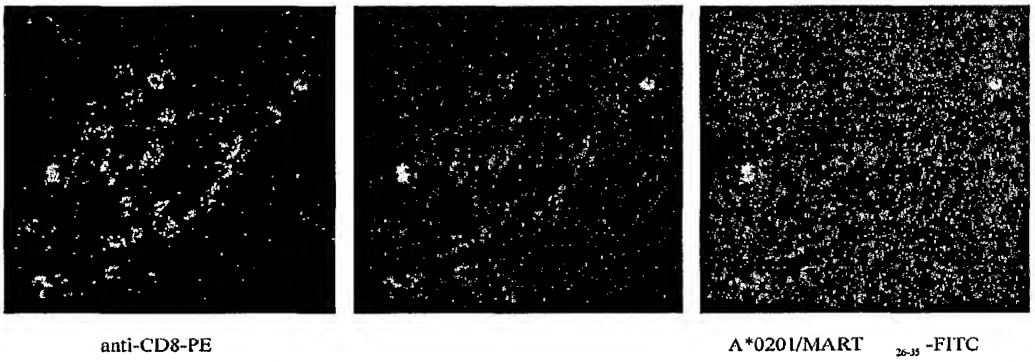


Figure 40

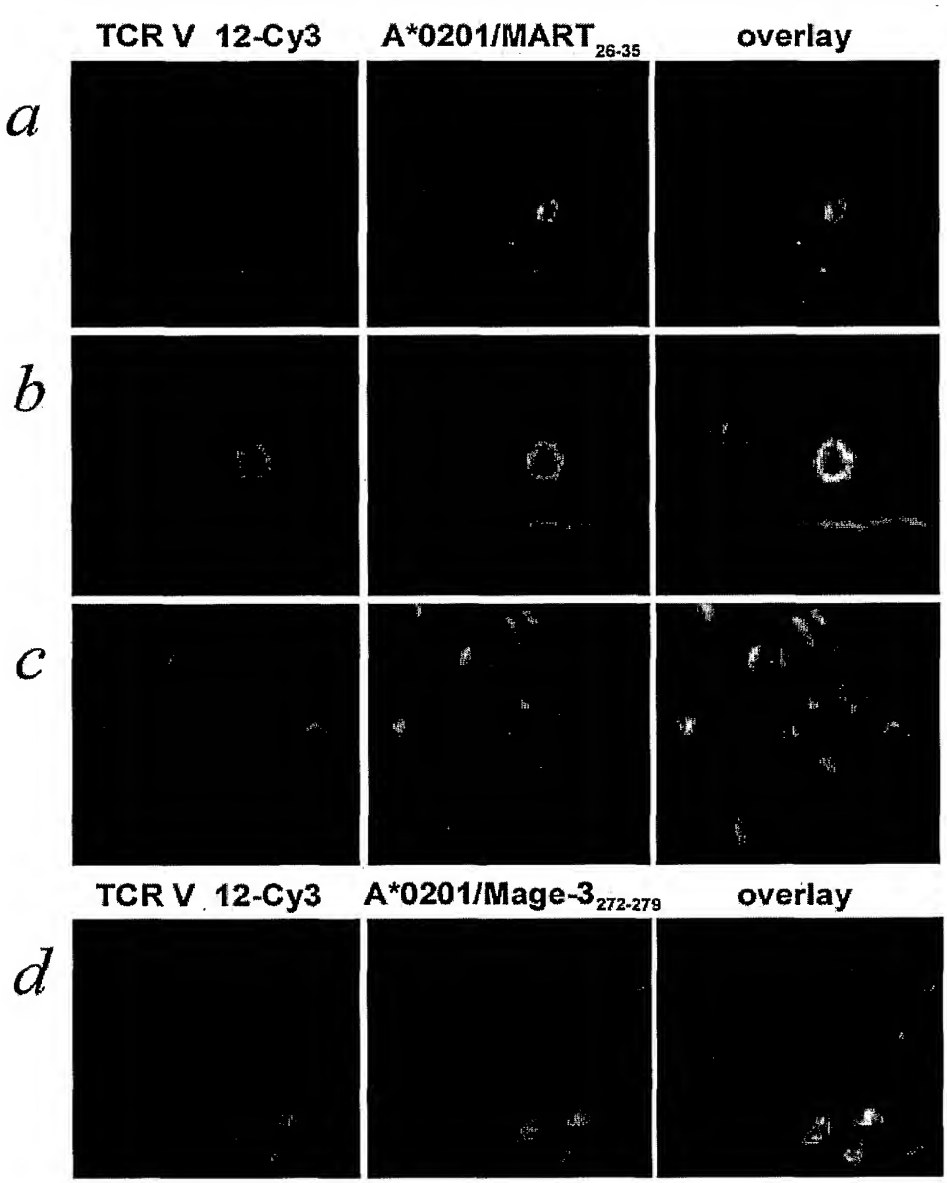

TCR V 12-Cy3
A*0201/MART26-35
overlay
a
b
c
TCR V 12-Cy3
A*0201/Mage-3272-279
overlay
d

Figure 41

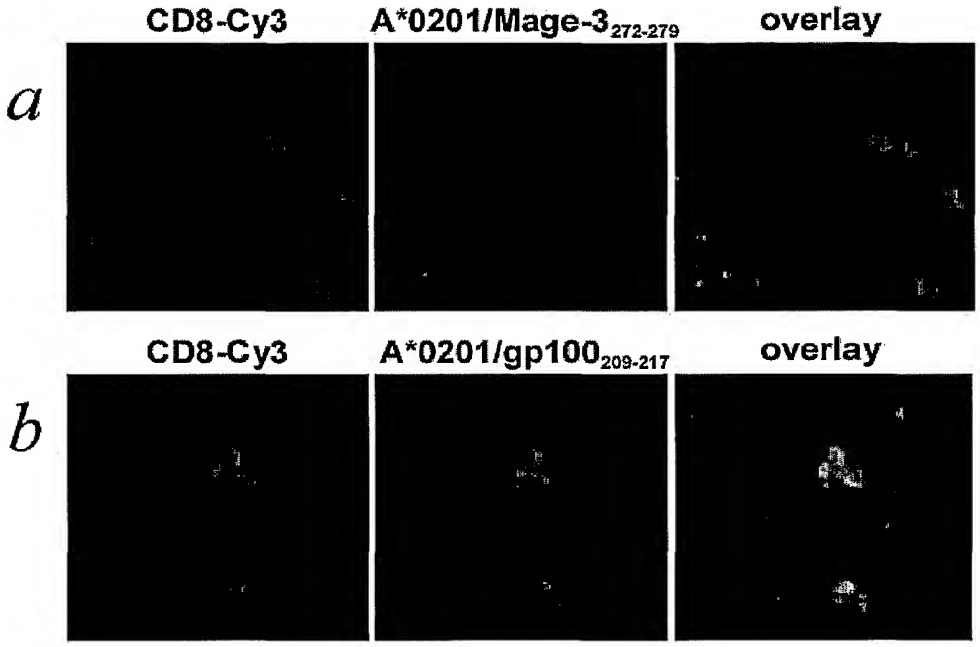


Figure 42

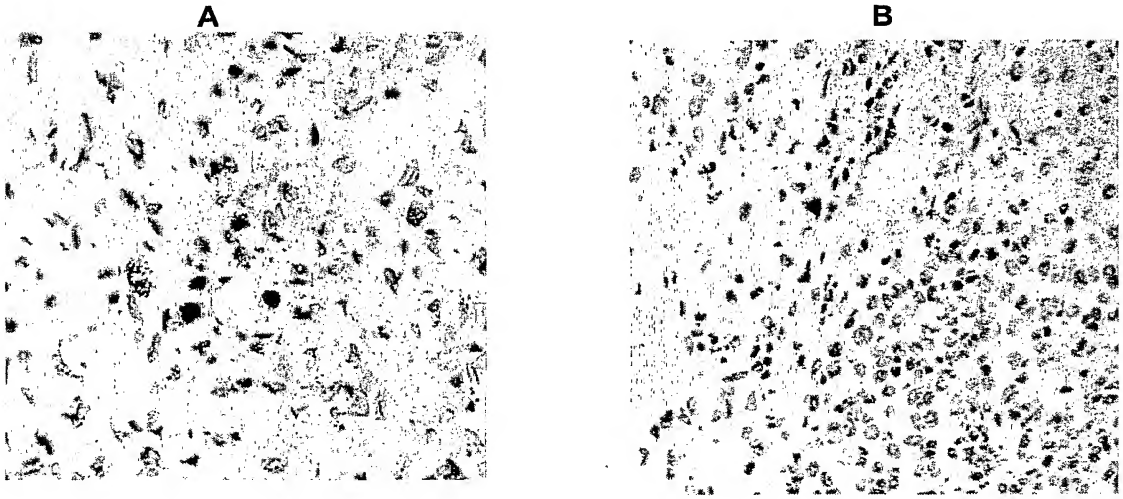


Figure 43

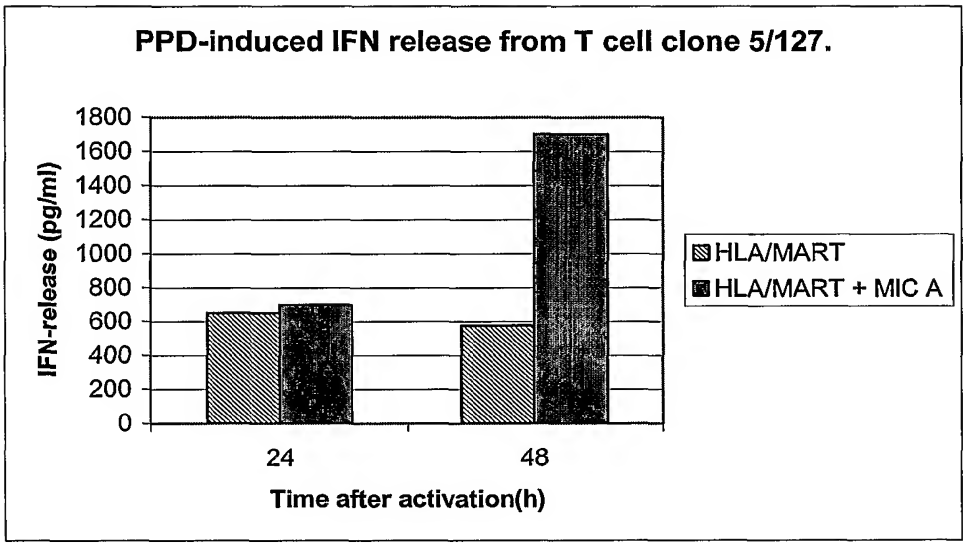


Figure 44

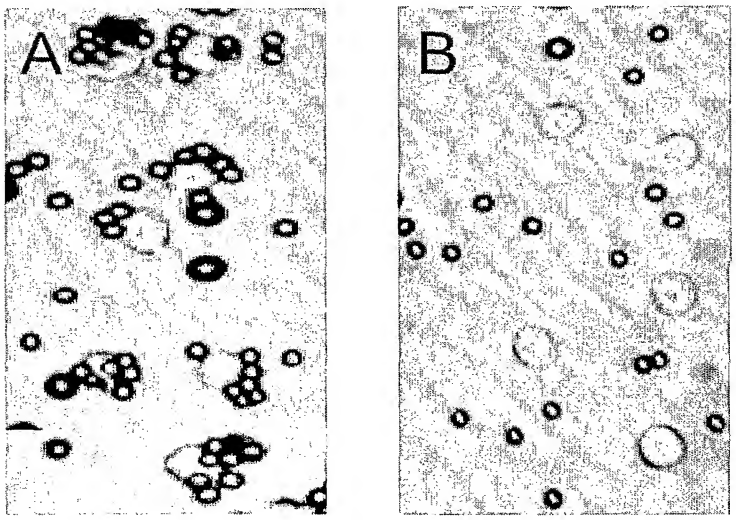


Figure 45

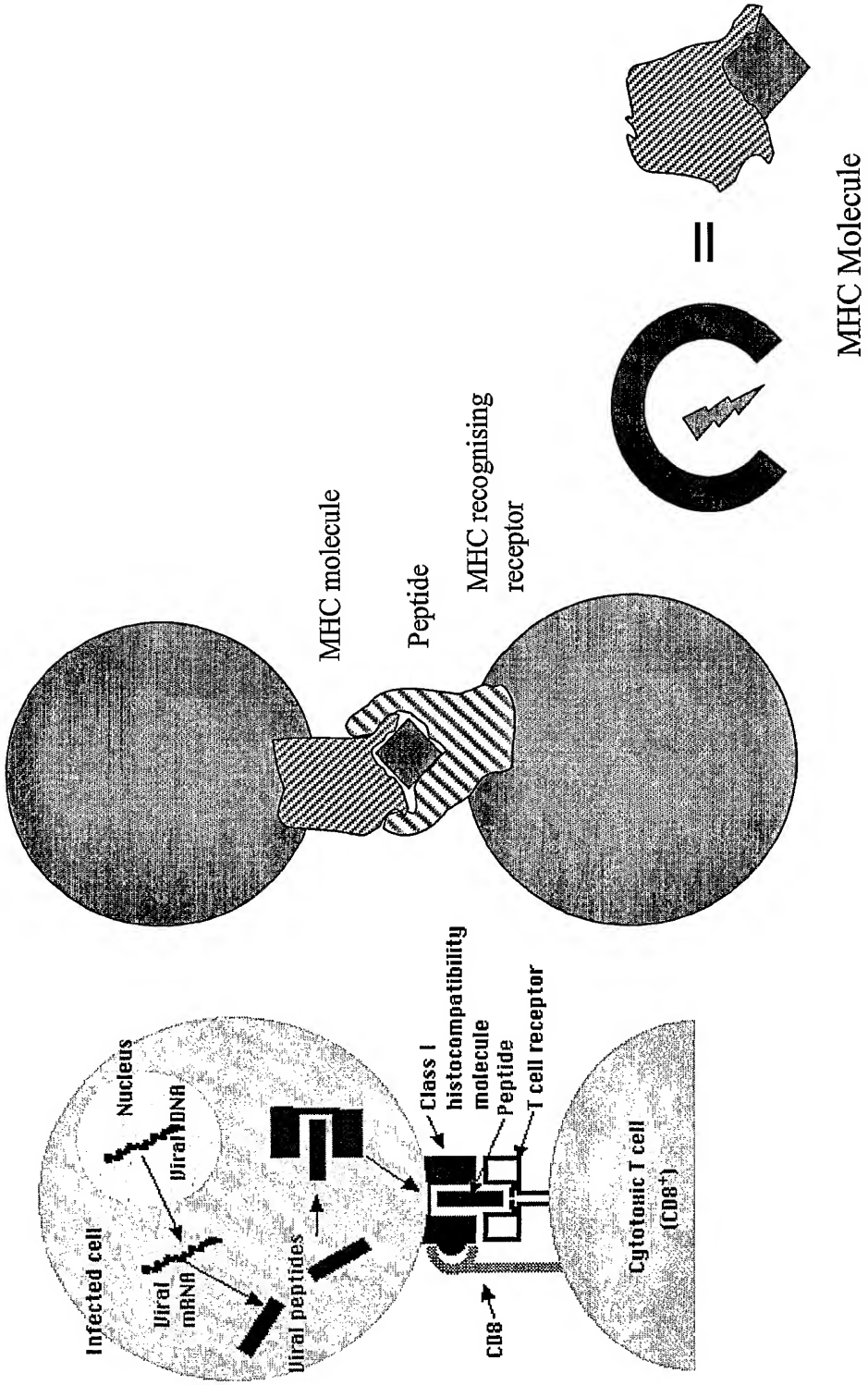

Infected cell
Nucleus
Viral DNA
Viral mRNA
Viral peptides
Class I histocompatibility molecule
Peptide
T cell receptor
CD8
Cytotoxic T cell (CD8+)
MHC molecule
Peptide
MHC recognising receptor
=
MHC Molecule

Figure 46

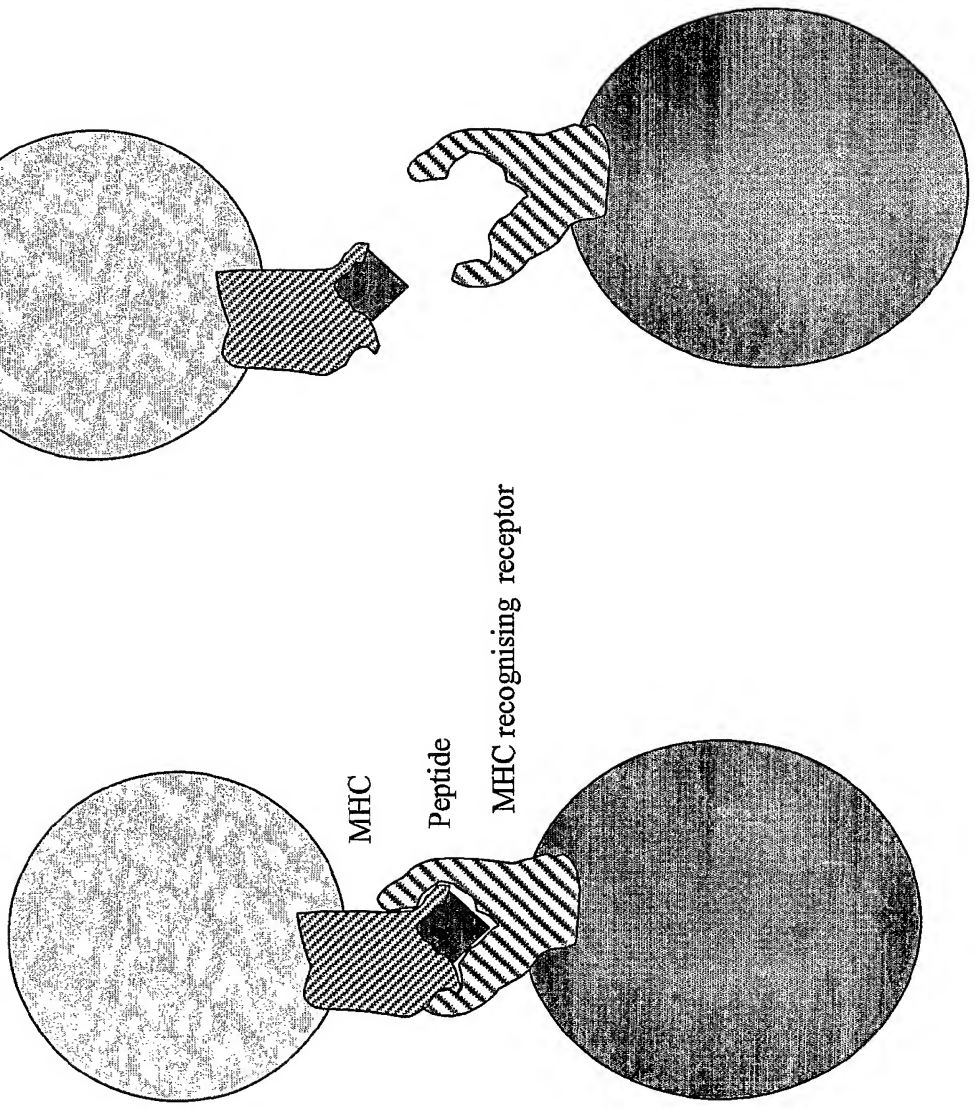


Figure 47

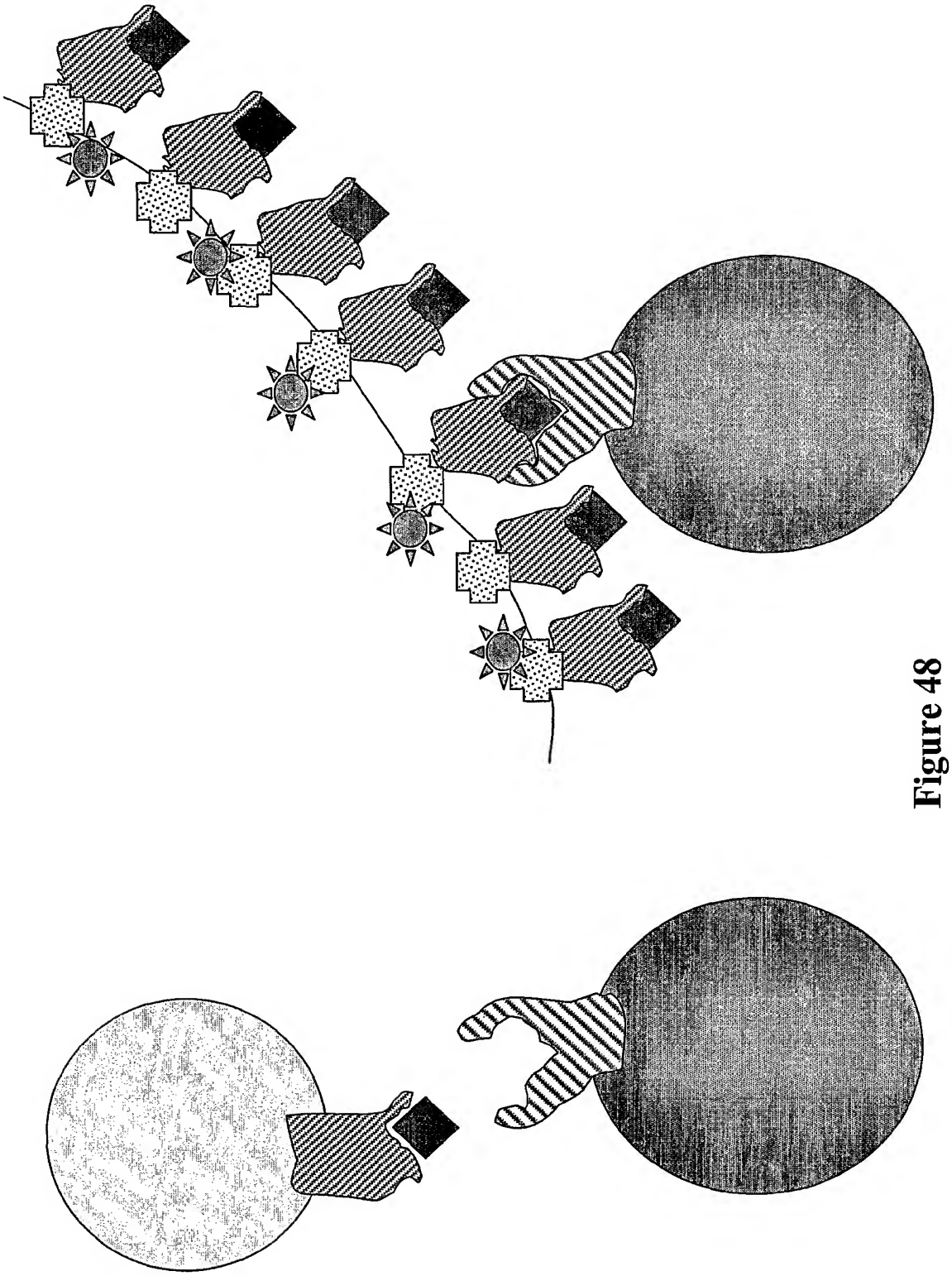


Figure 48

HLA molecules with peptides coupled to polymers on solid phase such as Dynabeads
Solid phase e.g Dynabeads
Polymer such as dextran
HLA class I/II with peptide V

Figure 49

HLA molecules with peptides and costimulatory molecules coupled to polymers on solid phase such as Dynabeads
Costimulatory molecules such as CD80/CD86
Solid phase e.g Dynabeads
Polymer such as dextran
HLA class I/II with peptide V

Figure 50

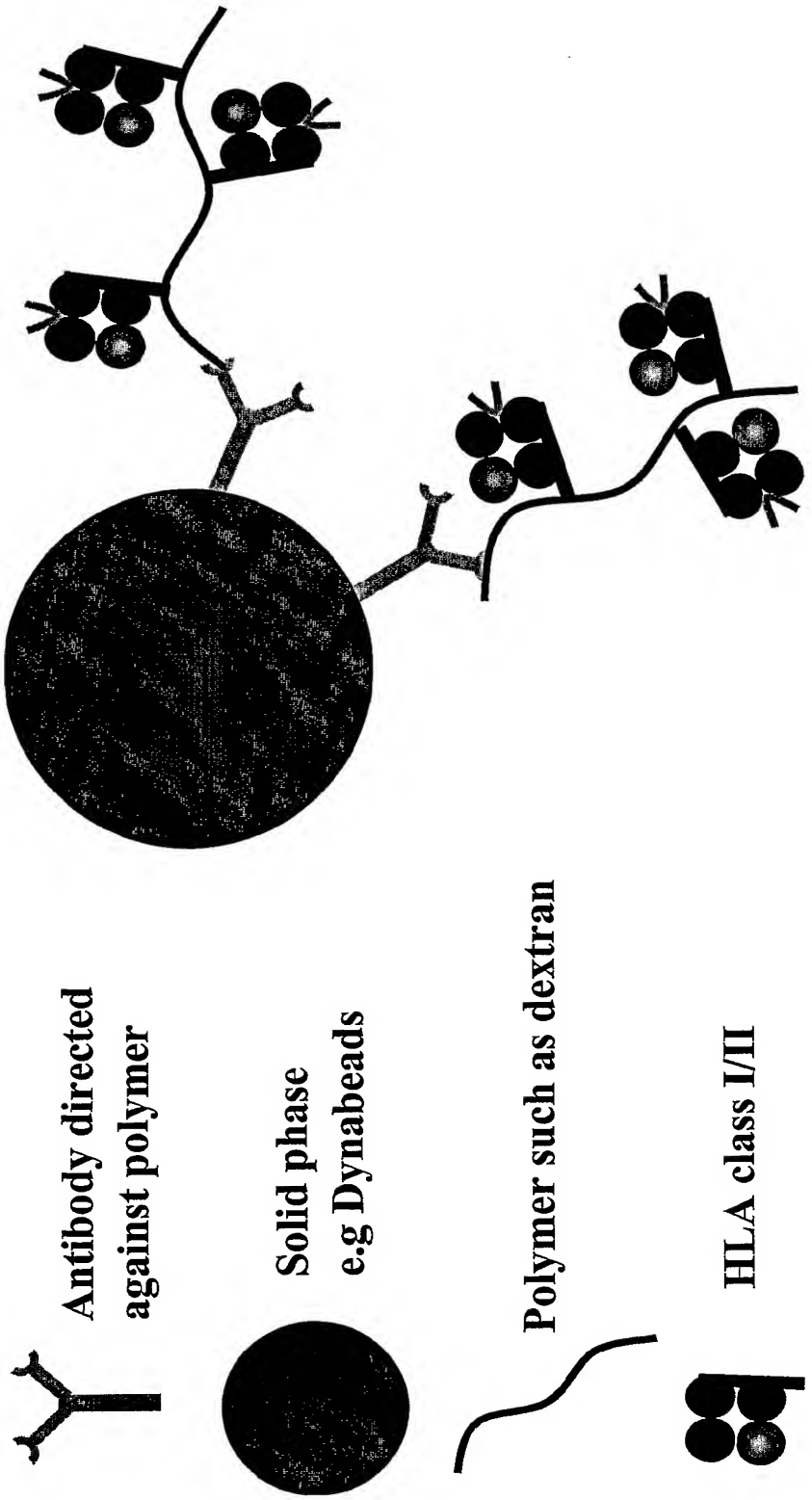

Antibody directed against polymer
Solid phase e.g Dynabeads
Polymer such as dextran
HLA class I/II
peptide

Figure 51

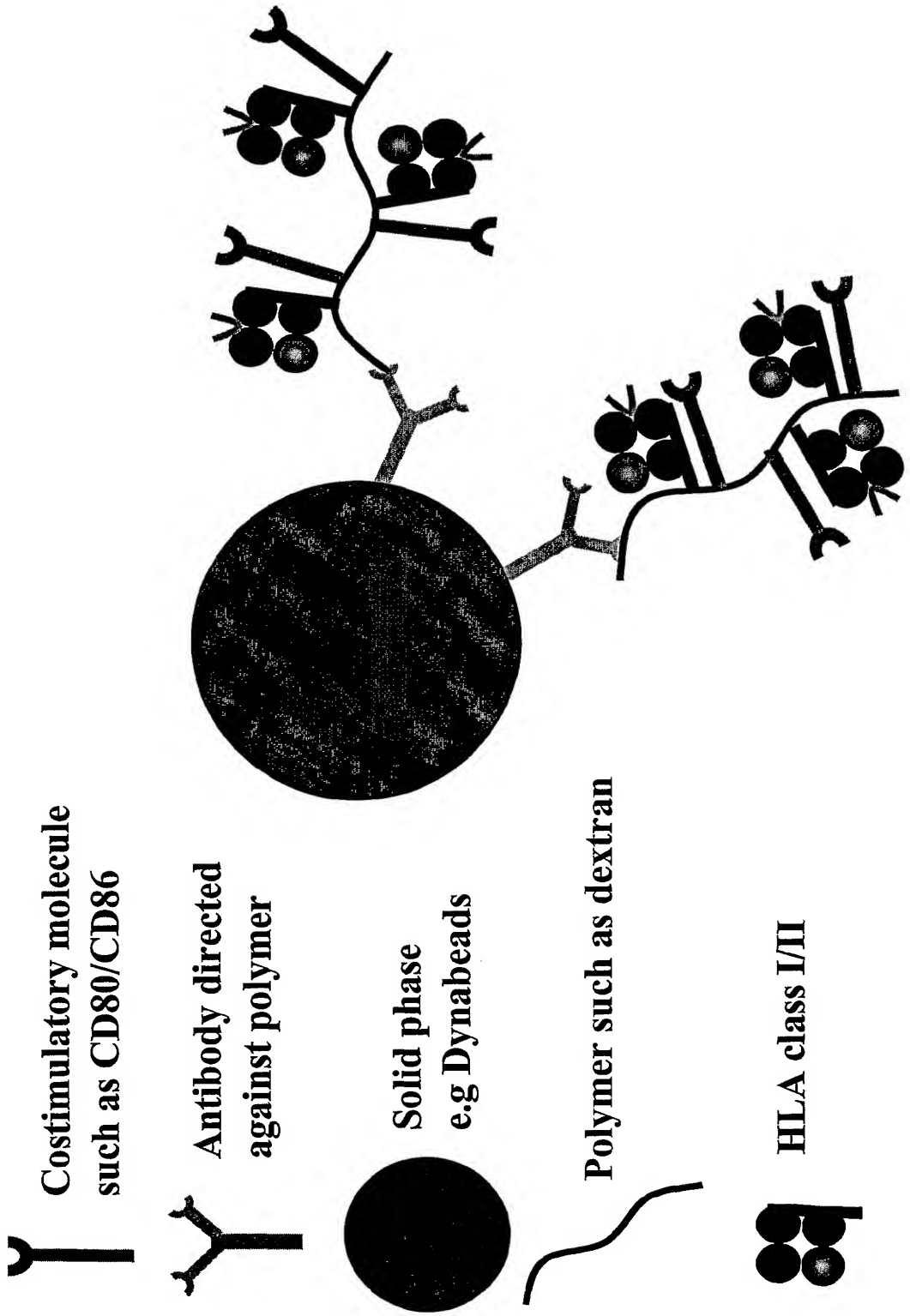

Costimulatory molecule such as CD80/CD86
Antibody directed against polymer
Solid phase e.g Dynabeads
Polymer such as dextran
HLA class I/II
peptide

Figure 52

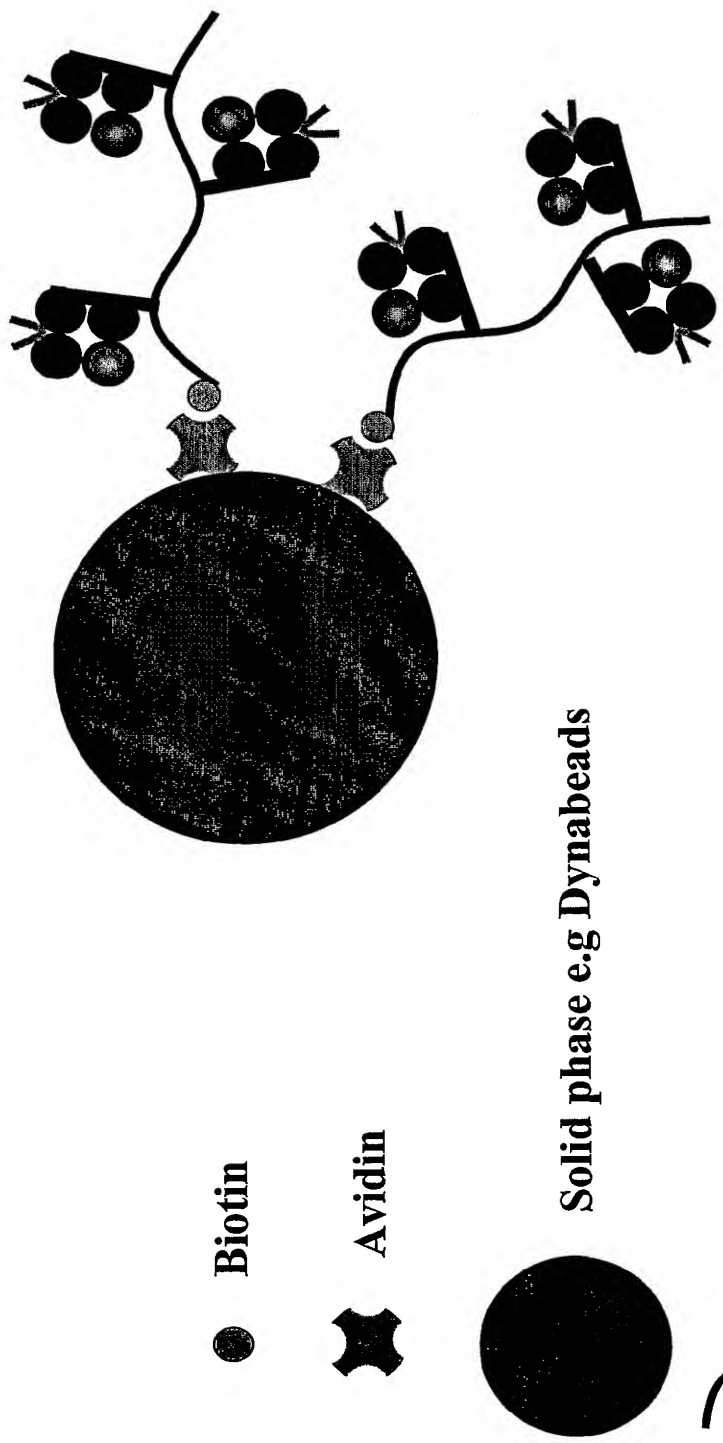

Biotin
Avidin
Solid phase e.g Dynabeads
Polymer such as dextran
HLA class I/II with peptide
HLA molecules with peptides coupled to polymers on solid phase such as Dynabeads via avidin-biotin

Figure 53

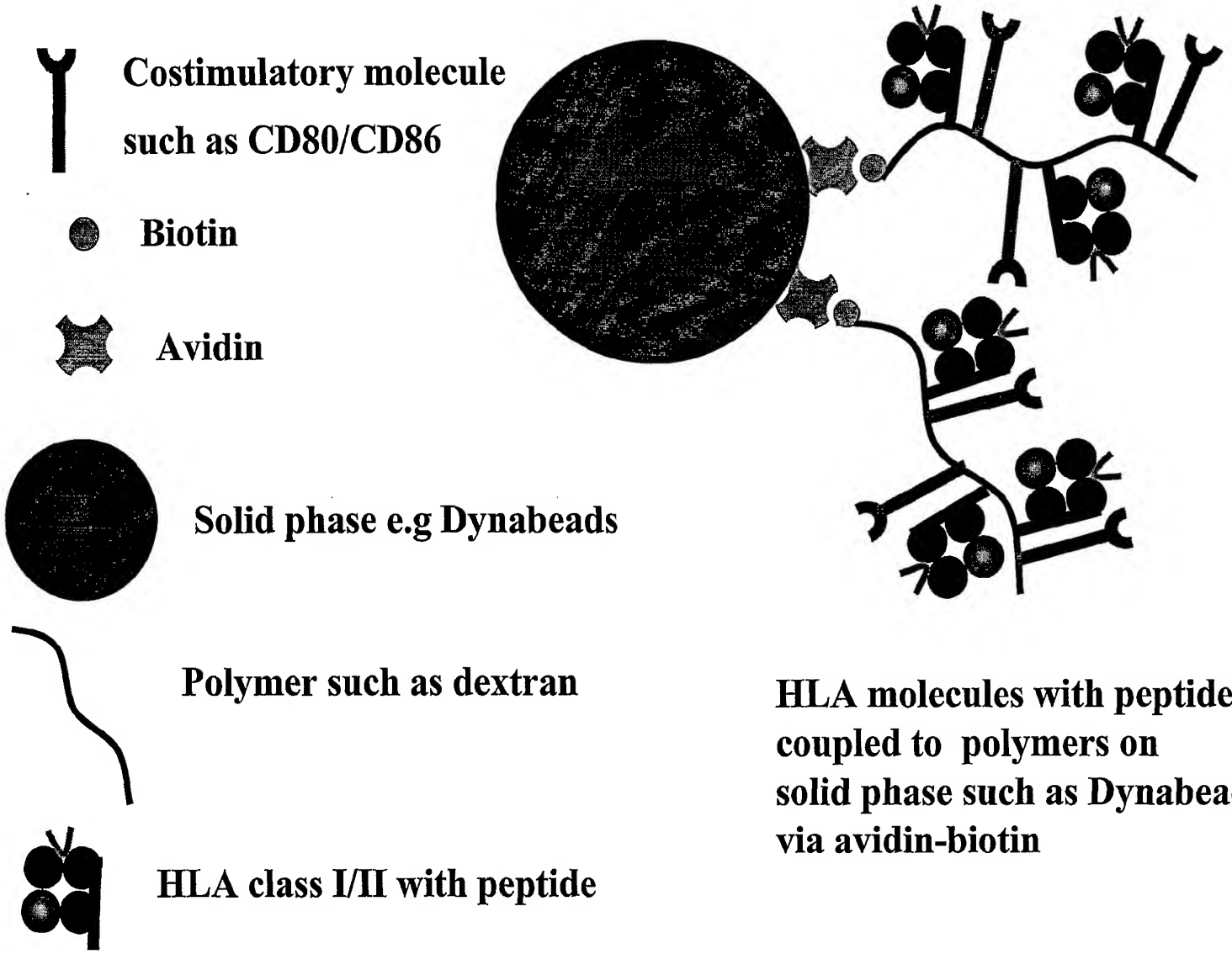

Costimulatory molecule such as CD80/CD86
Biotin
Avidin
Solid phase e.g Dynabeads
Polymer such as dextran
HLA class I/II with peptide
HLA molecules with peptides coupled to polymers on solid phase such as Dynabeads via avidin-biotin

Figure 54

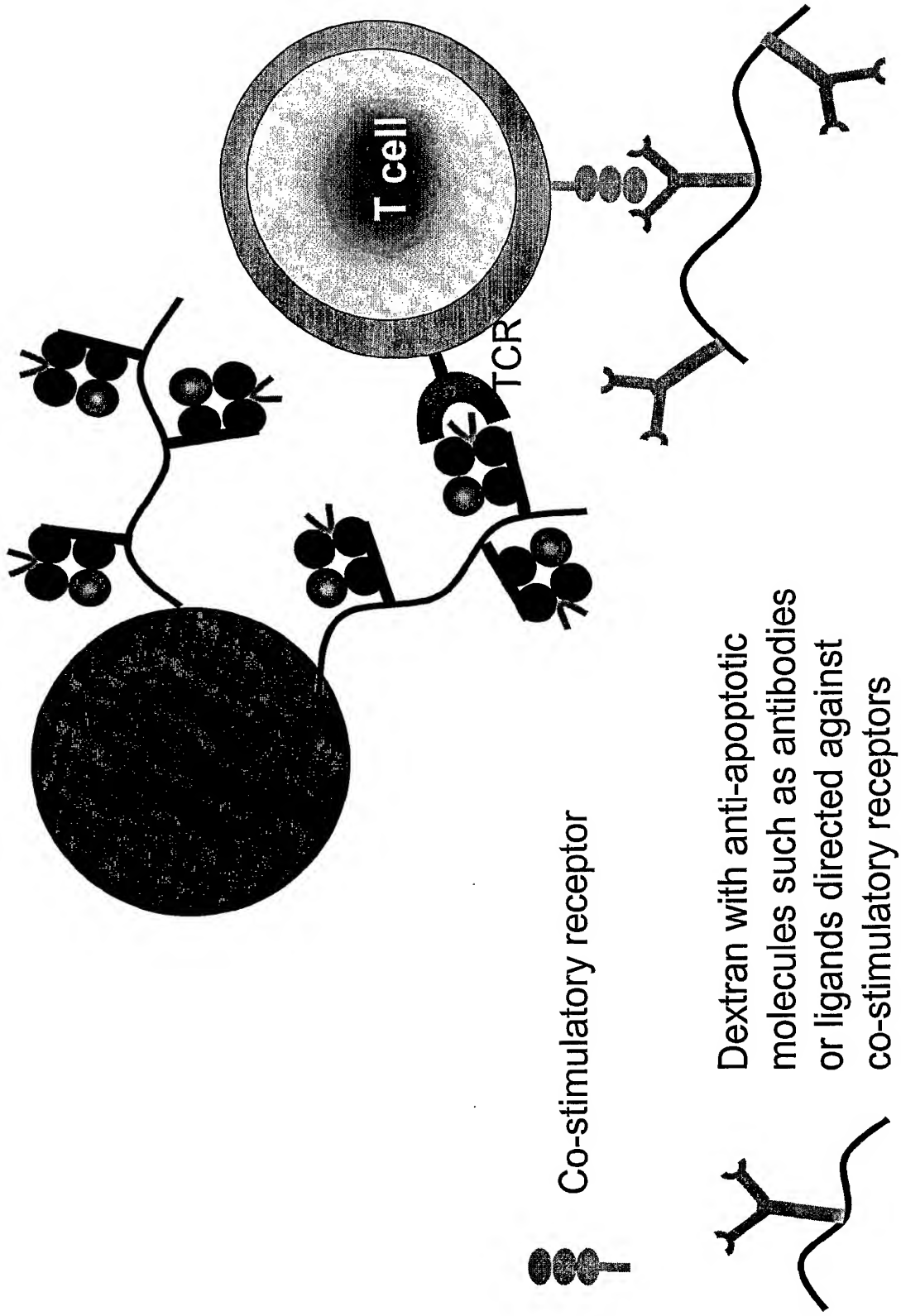

T cell
TCR
Co-stimulatory receptor
Dextran with anti-apoptotic molecules such as antibodies or ligands directed against co-stimulatory receptors

Figure 55

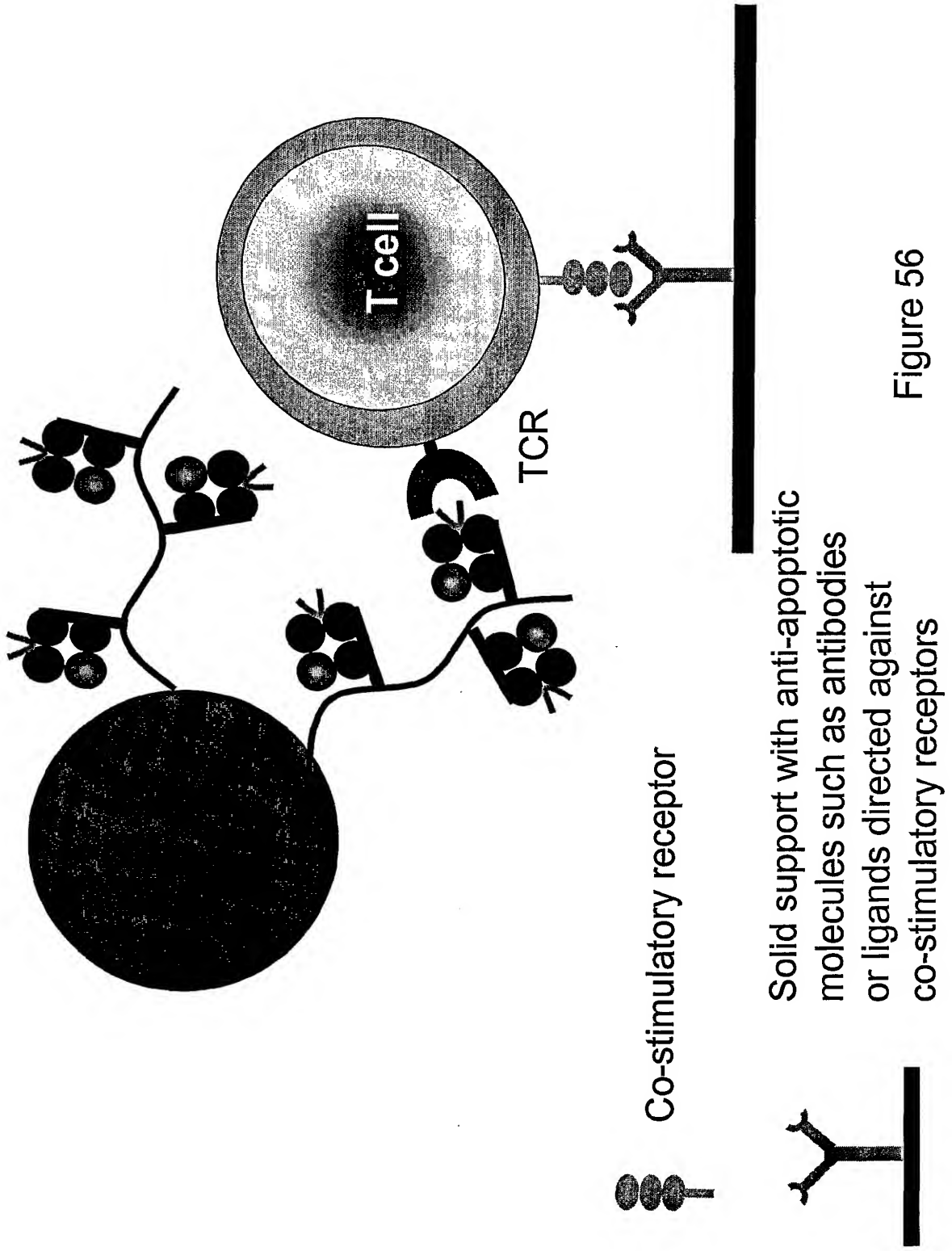

T cell
TCR
Co-stimulatory receptor
Solid support with anti-apoptotic molecules such as antibodies or ligands directed against co-stimulatory receptors

Figure 56

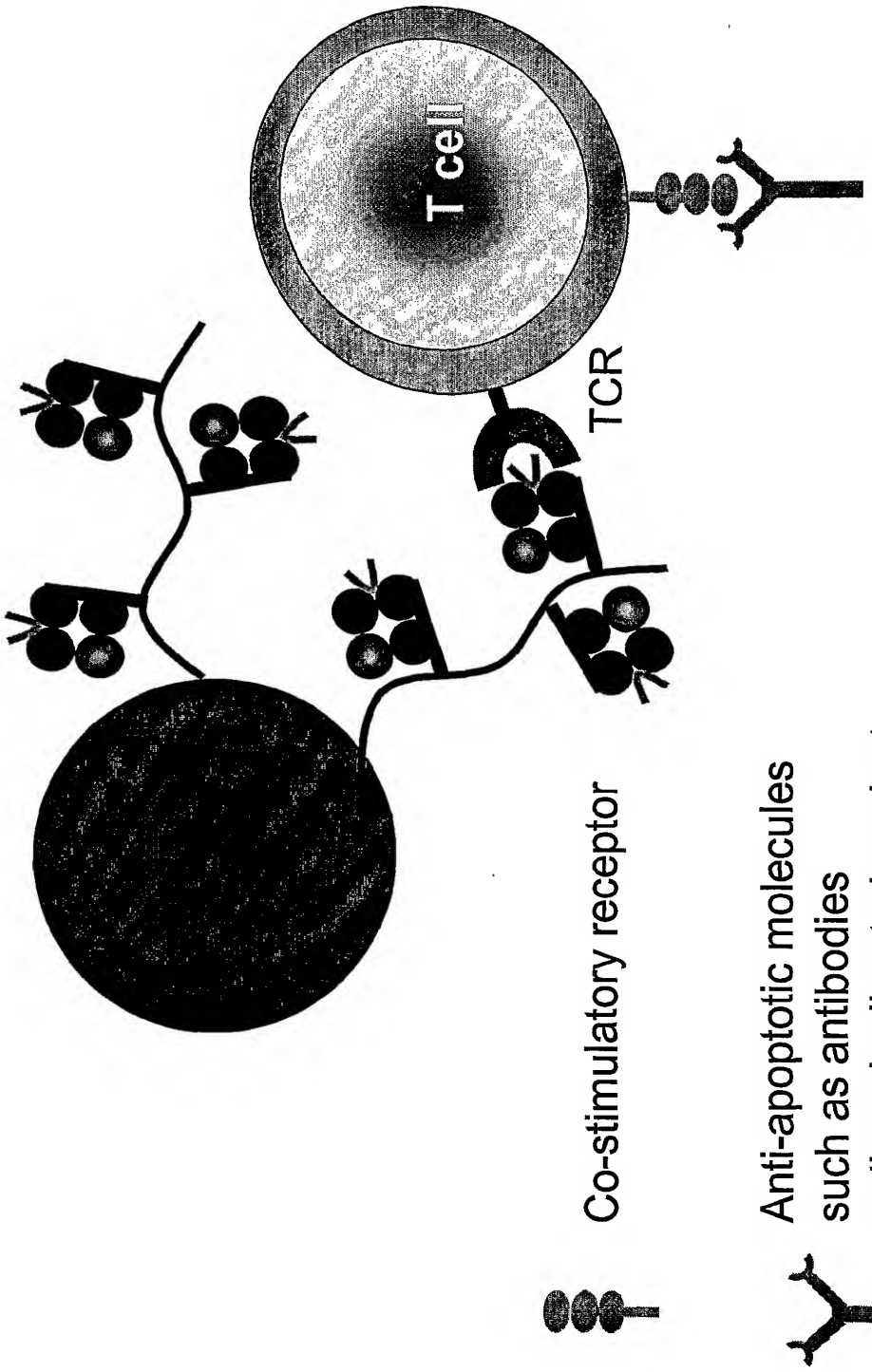

T cell
TCR
Co-stimulatory receptor
Anti-apoptotic molecules
such as antibodies
or ligands directed against
co-stimulatory receptors

Figure 57